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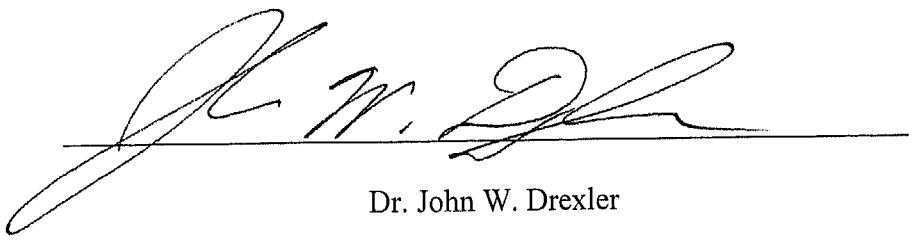

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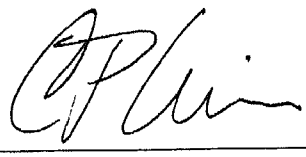

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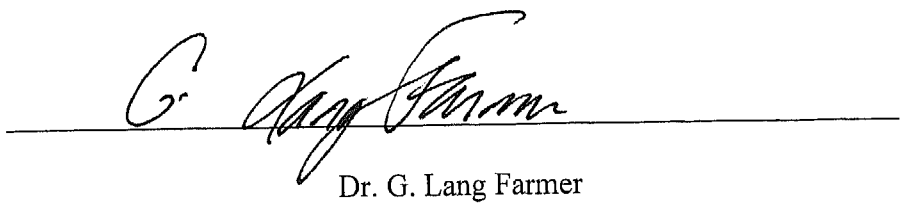

Dr. G. Lang Farmer

Date 4-28-97

Medlin, Elizabeth A. (M.S., Geology)

AN *IN VITRO* METHOD FOR ESTIMATING THE RELATIVE BIOAVAILABILITY OF LEAD IN HUMANS

Thesis directed by John W. Drexler, Ph.D.

Work to define the bioavailability of Pb from various sources is necessary to further establish the relationship between exposure and the harmful effects of Pb on humans. Most reliable "bioavailability" studies in this area have utilized animals or humans as subjects, which are costly and limited in their control. The proposed *in vitro* technique in this study is an alternative to those *in vivo* studies.

The main purpose of this research was to design an *in vitro* method to mimic dissolution of Pb in the stomach, and uptake in the small intestine. Heat, enzymes, stomach acids, and mechanical mixing, were ultimately used to quantify the bioavailability of Pb. This *in vitro* experiment has been calibrated to an accepted *in vivo* model, swine. Results showed a very close fit between the two data sets for Pb ($r^2 = 0.85$).

Other *in vitro* study parameters investigated with respect to their effects on the bioavailability of Pb were: pH, particle size, dose response, Pb mineralogy, and food presence in the stomach. Results showed that a decrease in either stomach pH or sample particle size may increase the bioavailability of Pb. Also, the physical and chemical make-up of a particular Pb sample had a dominant influence on subsequent bioavailability. These results substantiate the need for site-specific investigations.

This *in vitro* technique provides an inexpensive and easy way to thoroughly investigate each site for more accurate determination of acceptable Pb levels. In the

future, as more contaminated test samples are fed to the swine, more samples can be added to the regression line for the calibration of the *in vitro* method. Eventually this laboratory model may prove to be more efficient and cost-effective than *in vivo* models at predicting the bioavailability of soil lead in humans.

DEDICATION

"You will see by it, that the opinion of this mischievous effect from lead, is at least above 60 years old; and you will observe with Concern how long a useful Truth may be known, and exist, before it is generally receiv'd and practis'd on."

Ben Franklin, 1786

To my brilliant five-year-old niece, Victoria Marie Olmedo, in hopes that she and other women following after this time will be more encouraged to study the sciences than the women of my generation have been.

ACKNOWLEDGEMENTS

Many thanks to my parents, Jim and Sue, and to my sisters Margaret and Mary, for their tremendous support and love.

Thank you too, Andy for your undying faith and encouragement.

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GLOSSARY OF TERMS

in vitro - literally translated: "in glass" (Latin); refers to any non-whole, animal studies. Usually refers to removing an organ from an animal and testing it outside the body, however in this paper all components are synthetic.

in vivo - literally translated: "in the living" (Latin); refers to experimental studies that use animals as subjects.

availability - solubility or dissolution concentrations of a particular heavy metal in the human system; more specifically, the amount of Pb^{2+} in solution at equilibrium in the small intestine.

bioavailability - the portion of a heavy metal that is absorbed into the human bloodstream; is usually less than the "available" amount due to incomplete uptake in the small intestine.

***in vitro* relative bioavailability** - % BAF, calculated with the $[Pb_{(aq)}]$ analyzed in samples from *in vitro* experiment; can then be multiplied by a "percent uptake" from any system (human or animal) to get the "absolute bioavailability" of that system.

***in vivo* relative bioavailability** - calculated by comparing the reference material (lead acetate) to the test material (dosed soil-lead sample) to find the ratio of doses that produce equal biological responses in a test animal.

absolute bioavailability - the resulting percentage after an adjustment is made to the "*in vitro* relative bioavailability" amount by multiplying by an estimated "percent uptake" of a contaminant for a particular system.
For example, if the "absolute bioavailability" of lead in children is approximately equal to 50% of the "relative bioavailability" :

absolute bioavailability = 0.5 * (relative bioavailability).

I. INTRODUCTION

Lead (Pb) is nearly ubiquitous in our environment, enriched by both natural and anthropogenic sources. Once in the human body, Pb can cause many physiological problems. Today, Pb is the number one contaminant of concern according to the U.S. Environmental Protection Agency's (EPA) national priorities list of 275 toxins (Xintaras, 1992). It is generally thought that young (< 7 years old) children and women of child-bearing age represent the groups of highest risk from exposure to this contaminant. In 1995, the World Health Organization (WHO, 1995) called for further research in "work to define the bioavailability of lead from different sources and to establish the relationship between exposure and body burden". The proposed *in vitro* model presented in this paper aims at answering that call.

In this paper, the term *bioavailability* is used to describe the amount of a heavy metal that is absorbed into the human blood stream, and thus available for transfer to target organs such as the brain, liver, kidney, or bone. In contrast to this, the term *availability*, a precursor to bioavailability, refers only to solubility and dissolution concentrations, or to the amount of Pb that could be in solution at equilibrium in the small intestine. The resulting relationship between terms is the fraction of *bioavailable* lead is usually less than the *available* fraction, due to the

incomplete uptake of solubilized Pb by the small intestine (the principal organ involved in metal absorption). The term "*in vitro*", literally meaning "in glass", refers to any non-whole, out-of-body, animal studies.

The bioavailable fraction (BAF) of a particular contaminant is used as a conservative estimate of the uppermost exposure limit that may present a hazard to the high risk groups. Most reliable "bioavailability" studies use **animals** (e.g. **rat**: Barltrop and Meek, 1975; Jugo *et al.*, 1975; Dacre and TerHaar, 1977; Conrad and Barton, 1978; Mullen *et al.*, 1980; Aungst *et al.*, 1981; Dieter *et al.*, 1993; Freeman *et al.*, 1994; **swine**: LaVelle *et al.*, 1991; Casteel *et al.*, 1996; **monkey**: Freeman *et al.*, 1995; **rabbit**: Davis *et al.*, 1992; **frog**: Stansley and Roscoe, 1996; and **bird**: Connor *et al.*, 1994) or **humans** (Cleymaet *et al.*, 1991; Graziano *et al.*, 1996) as subjects; these have proven to be both costly and limited in their control. An *in vitro* procedure, when calibrated to an appropriate *in vivo* model, can be used as a less expensive, reliable technique for determining the bioavailability of lead in humans.

The purpose of this research was to design an *in vitro* method to mimic dissolution of heavy metals in the stomach and absorption in the small intestine. This simple, physiologically-relevant extraction procedure is used to estimate the bioavailability of lead in humans. As a necessary step, the *in vitro* test was calibrated to an appropriate *in vivo* (literally: "in the living", refers to using animals as subjects for experimental studies) model. For the purpose of this *in vitro* model, the swine has been chosen as an animal model that plausibly mimics the human digestive process (Weis and LaVelle, 1991).

Within its air and drinking water programs, the EPA often sets standard limits for particular toxins that would apply to human exposure. However, studies show that mineral characteristics, such as the variations in the physical and chemical lead species, may make it impossible for one standard to cover all soil Pb bioavailability (Barltrop and Meek, 1975; Cheng *et al.*, 1991; Cotter-Howells and Thornton, 1991; Davis *et al.*, 1992). If this is indeed the case, each situation can be tested individually by the use of this *in vitro* technique. For most risk assessment modeling for soil exposures, total concentrations of contaminants are used to estimate exposure risks in different environmental settings. However, when the importance of mineralogy, soil matrix, and particle size, are taken into account it may be determined that the acceptable soil Pb levels will be raised or lowered according to the lead mineral present at the site. Site specific data can be easily tested with the aid of an *in vitro* technique.

II. PROBLEM DESCRIPTION

2.1. Background

In 1991 the Centers for Disease Control and Prevention (CDC) recommended that the level of lead in blood (blood lead: PbB) not exceed 10 $\mu g\ dl^{-1}$ (Danse *et al.*, 1995); this is the same recommended level that stands today. This current level is contrasted to a much higher recommended PbB level from 1960 (just 31 years earlier) of 59 $\mu g\ dl^{-1}$ (Needleman, 1992). As research progresses and the public becomes more aware of the potential dangers of lead, this level may drop even further (Graziano, 1996). Most recently, this trend of concern over lead is evidenced by the citing of lead as a major hazardous chemical at 47% of the Superfund (National Priorities List or NPL) sites in the United States (Choudhury *et al.*, 1992). Schwartz (1994b) proposed that even a 1 $\mu g\ dl^{-1}$ decrease in the country's mean PbB level would produce as much as a $3.5 billion savings in annual health benefits costs.

Despite the potentially dangerous qualities lead possesses, it is also a useful metal to an industrialized society. Since the onset of the Industrial Revolution, lead use has undergone an exponential rise (Figure 1). The question of whether the pros

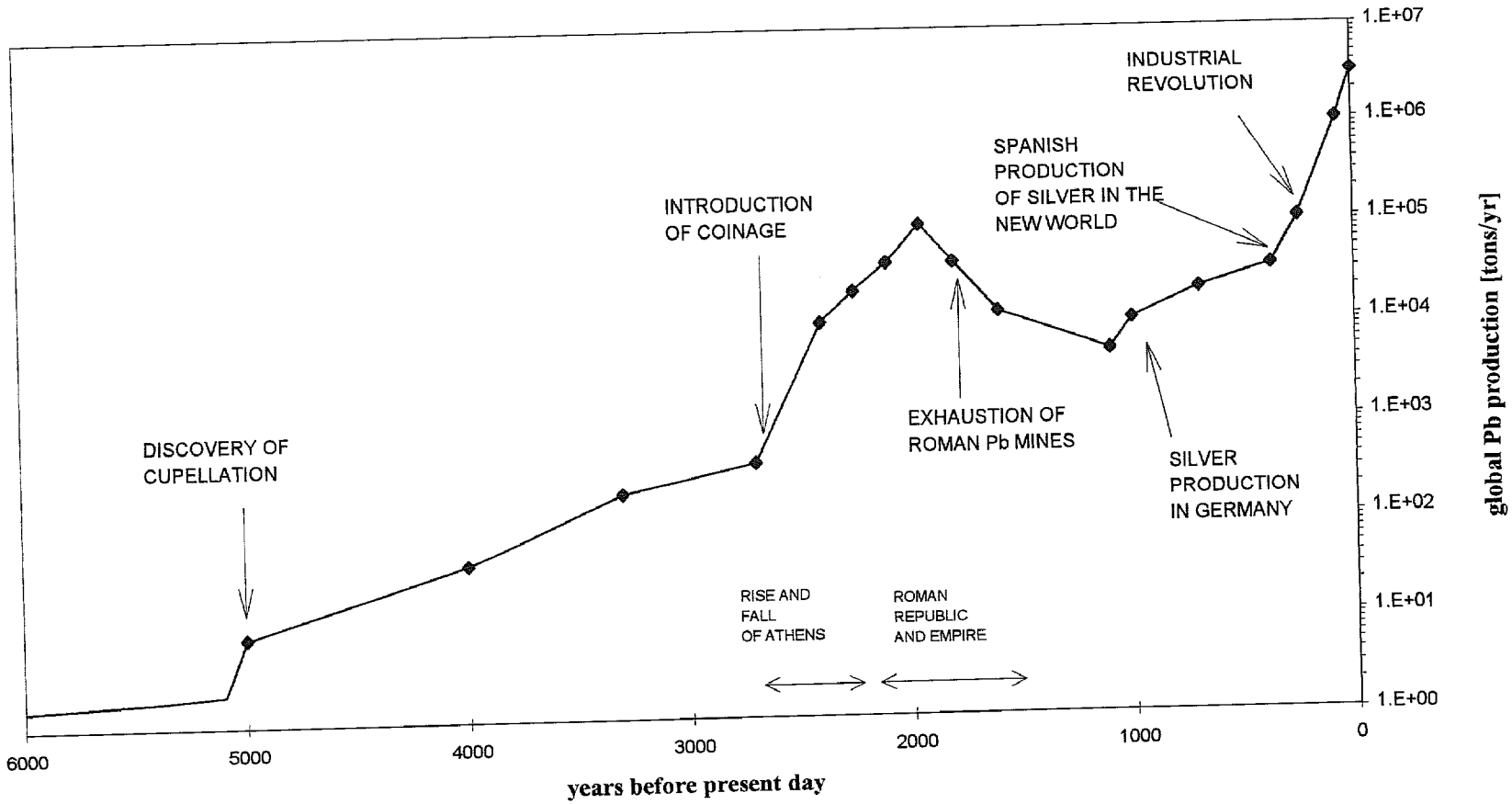


Figure 1. World lead production throughout history (notice logarithmic scale) (Patterson, 1980).

(usefulness for humans) outweigh the cons (harmfulness to humans) is still a matter of debate (Hays, 1992). Some researchers propose that there are alternative substances that could and *should* be used to replace Pb in many of its traditional uses because of the harm that environmental Pb causes to human health (Patterson, 1980).

The qualities that make lead such a useful metal are that it is exceptionally malleable, while also possessing high density, a low melting point, and a sound insulating quality. Lead also has a high corrosion resistance, and high opacity to gamma and x-ray energies (WHO, 1995). These qualities make for a metal that is very stable and easy to work with. Examples of common lead-sources encountered in daily activity are glass containers, batteries, solder, and chemical waste (Cheng *et al.*, 1991). In 1986, the United States used over 1 million tons of lead; storage battery production accounting for the bulk (76%) of this utilization (Matte *et al.*, 1992). Recently however, Pb concentrations in both gasoline (most lead was eliminated from gasoline in the U.S. by 1986; but leaded-gasoline is still a problem in undeveloped countries; Hafen and Brinkmann, 1996) and paint produced in the U.S. have significantly declined in response to more stringent environmental regulations.

2.2. Harmful Effects

The list of physiological complications resulting from Pb exposure is virtually endless – in fact, it is simpler to list those systems left unharmed by Pb exposure: skin

and muscle (WHO, 1995). The reason Pb is so toxic to so many physiological systems is that it interferes with a component of all cells, the mitochondrion (the energy-producing center of the cell). As a result of this interference, lead can then substitute for various essential minerals (Choudhury *et al.*, 1992).

Prior to acute lead toxicity, there are measurable physiological symptoms that can indicate possible lead exposure to the human system (Table 1). Blood lead (PbB) typically is the measurement for evaluating recent exposure in humans (Casteel *et al.*, 1996). Lead alters the enzymatic steps prior to haem (the Fe porphyrin component of hemoglobin) synthesis. One enzyme, ALAD (5-aminolaevulinate dehydratase) is inhibited by the presence of Pb in the system (at the 10 μg/100mL level). This inhibition, in turn, depresses the synthesis of another rate-limiting enzyme, ALA (5-aminolaevulinate). The consequence is that the precursors to ALA are over-produced, and then excreted in the urine (at the 40 μg/100mL level).

Table 1. Whole blood lead levels associated with specific biological responses in children (National Academy of Science, 1980).

Blood Lead Level (μg Pb/100 mL)	Effect
8	Subtle neurological impairment (Graziano, 1996)
10	ALAD inhibition
15-20	Erythrocyte protoporphyrin elevation
40	Increased urinary ALA excretion
40	Anemia (lowered hemoglobin count)
40	Coproporphyrin elevation
50-60	Cognitive (CNS) deficits
50-60	Peripheral neuropathies
80-100 (acute levels)	Encephalopathic symptoms (also occur at low levels)

Lead also interferes with the conversion of protoporphyrin to haem by inhibiting ferrochelates (15-20 μg/100mL level). Another enzyme couple that is inhibited by Pb is COPRO-O/COPRO, with the over-production of COPRO (coproporphyrin) also detected at the 40 μg/100mL level. Lead-induced anemia (40 μg/100mL level) can also be caused directly by the inhibition of haem production. Anemia is a common effect seen in occupationally-exposed lead workers that is easily diagnosed and thus a clear lead toxicity marker.

As the haem body pool is depleted by Pb, there is a potential for a multi-organ impact such as: cardiovascular dysfunction; impaired development of the nervous system (50-60 μg/100mL level); peripheral neuropathy (often detected by weakened limbs); impaired bone and tooth development; and at the 80-100 μg/100mL level, elevated brain levels of tryptophan, serotonin, and HIAA.

With increasing blood lead levels, the consequences to the human body become more grave; though the subtle effects of low Pb concentrations may also significantly contribute to possible neurological disorders observed in children (Needleman and Gatsonis, 1990). Before lead can harm the body's cells, it must be absorbed and transported to target organs like the brain, liver, kidney, or bone (Fowler and DuVal, 1991; Dieter *et al.*, 1993). Many questions still remain as to the precise mechanisms of Pb toxicity in humans. No single mechanism seems to be sufficient to account for all diverse effects of Pb (Luthman *et al.*, 1994).

Although data are sparse concerning the effects of lead on the immune system, animal studies have shown Pb to be a depressor of the immune system (Lutz *et al.*,

1994). Examples of detrimental effects of Pb in children are: impaired growth and hearing; possible reduction in IQ; reduced hemoglobin synthesis; reduced vitamin D metabolism; and reduction in nerve conduction velocity. Other signs of Pb toxicity are: increase in erythrocyte protoporphyrin; impaired kidney function; colic; anemia; encephalopathy; and even death. (Mahaffey, 1977; Xintaras, 1992). Adults can experience many of the same effects from lead exposure as those seen in children. Typically, adults experience peripheral neuropathies prior to CNS effects. Adults may also undergo an increase in blood pressure (Khalil-Manesh *et al.*, 1994), infertility, and shortened life span (Xintaras, 1992).

By using the markers in Table 1, blood-lead levels can be used to assess potential lead toxicity in humans. According to the EPA, blood lead is a more appropriate test for exposure to lead, than a level in air or an oral level (Choudhury *et al.*, 1992). However, the half-life ($t_{1/2}$) of lead in the blood is about 36 days, so it can represent only recent exposures (WHO, 1995). Most Pb is stored in bone: about 90% in adults, and 70% in children (Steenhout, 1992; Dieter *et al.*, 1993). The bone continues to be an endogenous source of lead to the body throughout a lifetime (Steenhout, 1992). Teeth also have a high capacity for absorbing lead from solution, and may prove useful in measuring the internal Pb exposure over longer periods of time (Cleymaet *et al.*, 1991; Karakaya *et al.*, 1996). Estimation of the amount of lead remobilized from these endogenous sources due to the processes of growth or osteoporosis for example, have been measured by long-term stable isotope analysis (Gulson *et al.*, 1996b).

2.3. Lead in the Environment

For cationic metals, the ion is the primary bioavailable form (Hamelink *et al.*, 1994). For lead, the Pb^{2+} ion is the dominant environmental form (Davies, 1990). Environmental lead exposure is possible from many different pathways, such as: air (e.g. smelter activity, ongoing automobile emissions in developing countries; EPA limit: 1.5 $\mu g\ m^{-3}$); food (e.g. canned food, and seafood); water (e.g. lead pipes, solder, and brass taps; EPA limit: 15 $\mu g\ L^{-1}$); indoor housedust (the EPA is currently setting a limit for interior dust and bare soil under Title X of the Housing and Community Development Act of 1992; Clark *et al.*, 1996); and soil. Soil particulate matter contains a variety of possible binding sites for lead (Table 2).

Table 2. Classification of Pb binding sites in environmental media (Chaney *et al.*, 1989).

Classification	Example
Surface absorption	Amorphous crystalline Fe and Mn oxides. Clay minerals.
Organic matter	Uptake by living organisms. Chelation/complexation.
Lattice	Secondary minerals. Undecomposed primary minerals.

An example of a lead form found in soil is $PbSO_4$ which has been shown to form from the oxidation of PbClBr found in gasoline emissions (Harrison *et al.*, 1981). A recent study site adjacent to an interstate (I - 275, Tampa, FL) showed over

50% of the samples (n = 224) had Pb levels of 500 μg g^{-1} or greater. Unusually, they did *not* find evidence for decreasing Pb content with distance away from the highway (Hafen and Brinkmann, 1996). Other prevalent environmental forms of lead are the lead oxides, carbonates, and sulfates found in older house paints. Lead oxides are particularly toxic due to their high solubility in acidic medium. Environmental lead contamination is also found associated with sewage sludge and lead-arsenate insecticides (Engel *et al.*, 1996).

Examples of heavily contaminated sites with high concentrations of lead are: Leadville, Colorado, from turn-of-the-century mining operations (Levy *et al.*, 1992); Port Pirie, Australia, the world's largest and oldest primary Pb smelter, processing ore mainly from the Broken Hill deposit (Calder *et al.*, 1994; WHO, 1995); Derbyshire, England, where lead contamination remains from 2000 years ago during time of Romans (Cotter-Howells and Thornton, 1991; WHO, 1995); and Arima, East Trinidad, a present-day battery recycling and lead smelter waste site (Mohammed *et al.*, 1996).

2.4. Routes of Entry - Exposure Pathways

An important issue in isolating the effects of lead exposure is finding and quantifying the route of entry of Pb from the environment to humans (Figure 2). The main pathways for exposure between Pb and humans are: water, soil, dust, and the

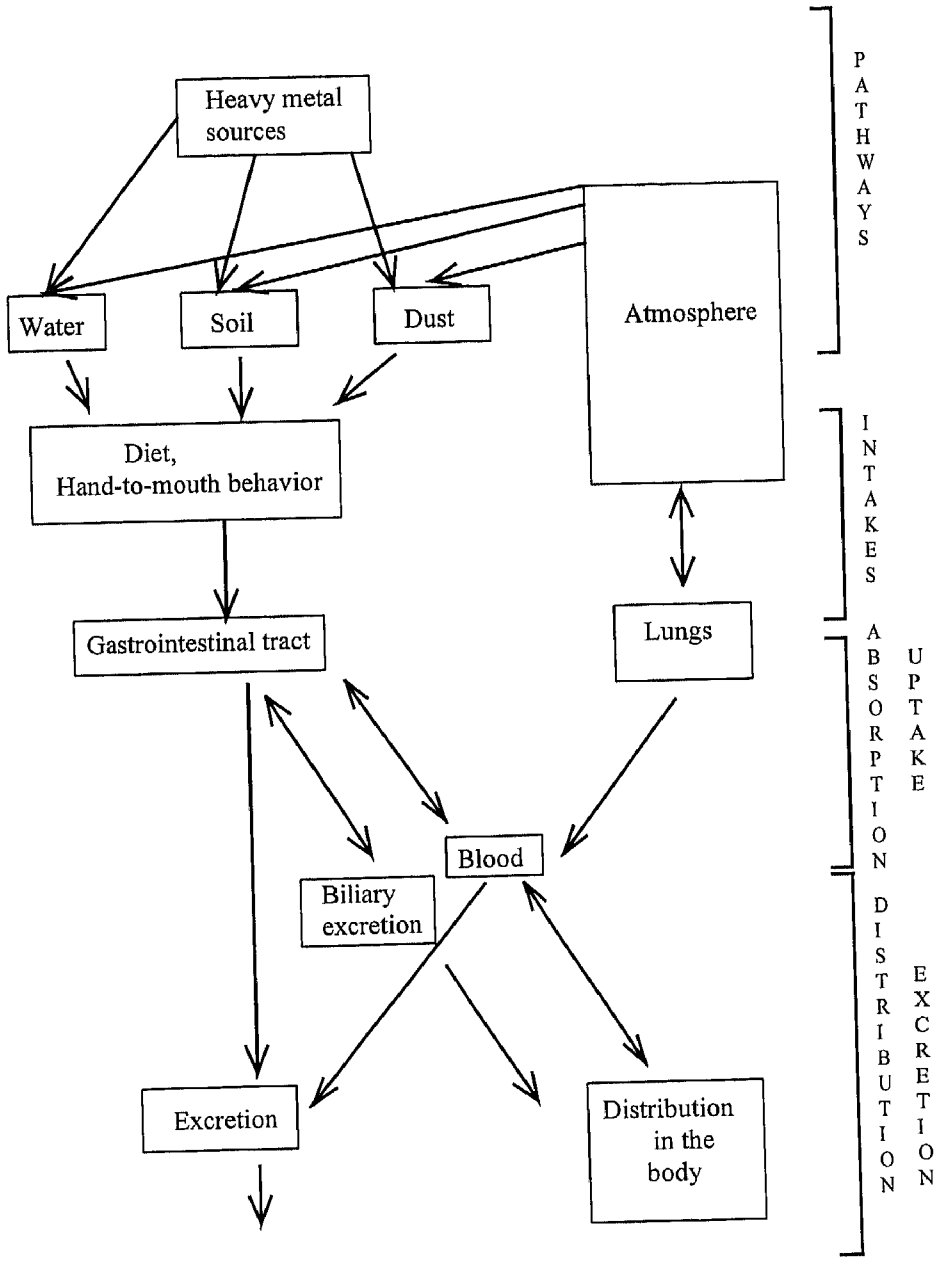


Figure 2. Routes of lead uptake and distribution in the body (Modified from Fergussen, 1990).

atmosphere. Intake, following exposure, can occur via diet or hand-to-mouth behavior. The two main routes of intake of inorganic lead particles are either through the lungs (inhalation), or through the gastrointestinal tract (ingestion). For the *in vitro* experiment presented in this thesis, the gastrointestinal tract is examined as the primary route for lead entry into the body (uptake) (Figure 3). Once Pb intake has been achieved, then the Pb is absorbed into the blood stream and then distributed or excreted from the body. Compartments within the body that may accept Pb from blood are the bone, kidney, liver, and brain.

Environmental sources of lead such as contaminated soil, mining waste, industrial waste, and paint, are typical sources of Pb exposure to humans (Figure 4). Incidental soil ingestion has often been identified as one of the most significant routes of exposure affecting final risk assessment estimates for children (Calabrese and Stanek, 1995). Hand-to-mouth behavior, such as thumbsucking, nail-biting, and mouthing of nonfood items, will increase the likelihood of soil ingestion by young children (Bornschein *et al.*, 1986). Lead present in the home is also a concern. Butler and MacMurdo (1974) have shown that the Pb concentration in housedust will tend to equilibrate with the outside airborne Pb, whether or not it is tracked in by the residents. The EPA lead model, IEUBK, contains a default assumption for indoor dust lead concentration equal to the outdoor soil Pb (or it is equal to 70% of the outside soil plus an additional contribution from airborne lead). The relationship between indoor and outdoor lead concentrations may vary significantly depending on other factors such as, presence of indoor lead paint or lead-based hobbies (Tsuji and Serl, 1996). During the wintertime months, most children spend more of their time

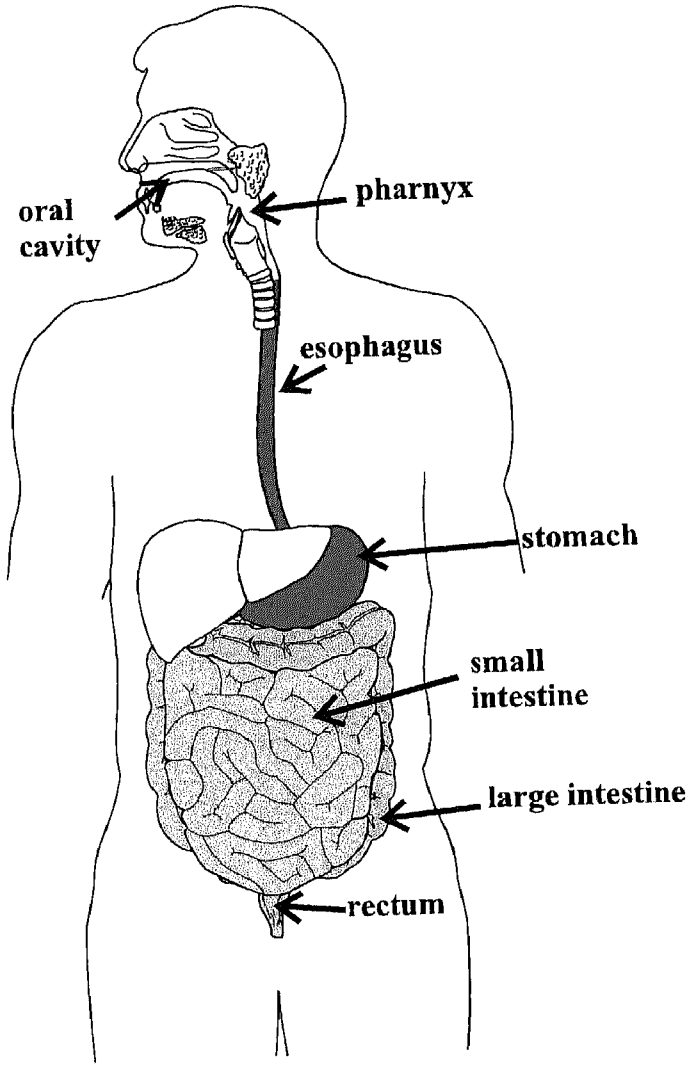


Figure 3. Diagram of human anatomy of gastrointestinal tract.

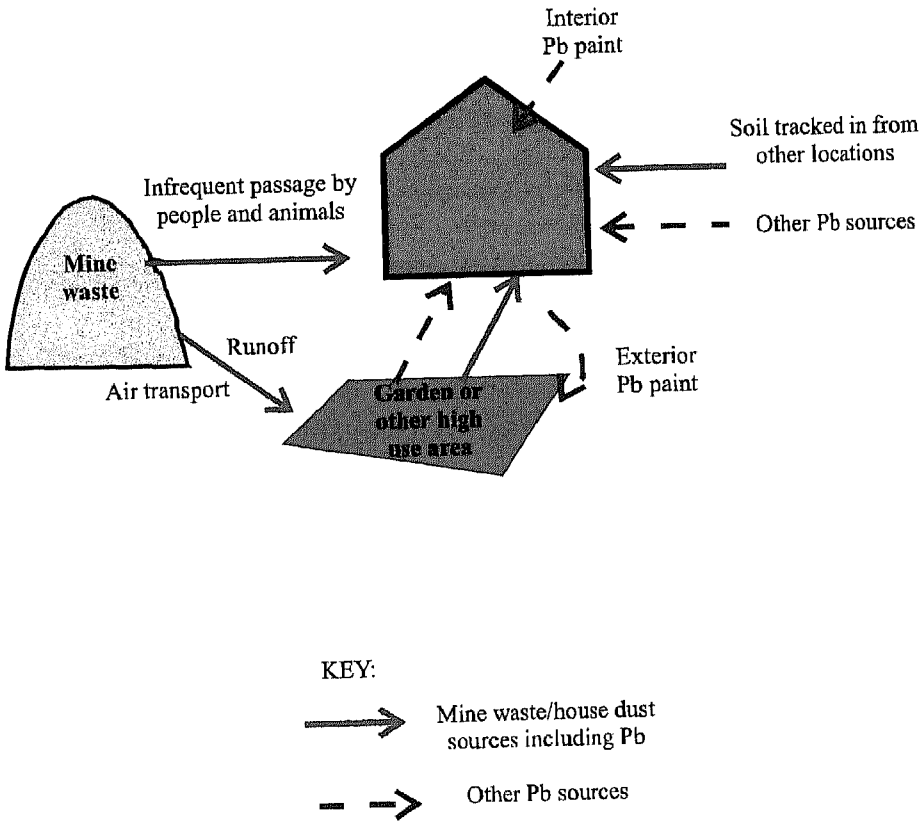


Figure 4. Sources of lead in mining community (Steele et al., 1990)

indoors, so the presence of Pb-contaminated house-dust is an important consideration as it seems that the home may offer little or no protection.

Exposure to lead at the workplace is also a major route of consideration. Many jobs and hobbies involve the use or manufacture of lead products. Examples of occupations that involve lead exposure are: enamelers, ceramic or pottery crafters, glass blowers, jewelers, demolition workers, paint removers, battery makers, insecticide makers, plumbers, and miners or lead smelter workers. An alarming 76% of workers at smelter operations have PbB > 60 $\mu g\ dl^{-1}$. Of adult males with elevated PbB, surveys showed that 98% of them were potentially exposed to lead at work. Another documented exposure route for lead workers is the "fouling of one's own nest". This phrase refers to the practice of lead workers unintentionally transporting the lead particulate home with them, thus increasing the risk of lead exposure to their families (Matte *et al.*, 1992).

2.5. Mechanisms of Biological Uptake

The complex process of metal uptake by the human body via the small intestinal membrane can be understood as a three-stage process: adsorption onto the membrane wall, transfer through the cell membrane, and movement into the plasma (Ragan, 1983). Other processes that may facilitate lead absorption in the body are:

diffusion through tight junctions; and pinocytosis – neither of which require Pb interaction with the membrane.

The first stage in membrane transfer involves metal uptake into the mucosal cell. The efficiency of adsorption depends on: the affinity of the Pb ion for tissue binding sites; availability of binding sites on the intestinal wall; and the nature of interaction between Pb^{2+} and intestinal tissue (Blair *et al.*, 1979).

The second step in metal uptake is transfer through the cell membrane. Metals may be too hydrophilic to diffuse across lipid bilayers. Due to this phenomenon, transport of metals across biological membranes is often mediated by organic ligands (Figure 5).

Transport of complexes through the membrane to the blood can be either active or passive transport (Mushak, 1991). Passive transport occurs due to simple diffusion following a concentration gradient imbalance. Active transport is an energy-requiring process fueled by the maintenance of chemical gradients. In the active transport process, the membrane concentrations of elements can be maintained in disequilibrium with the expenditure of energy (i.e., ATP).

Carrier-mediated diffusion is a type of passive transport. Most metal ions are absorbed by carrier-mediated processes. The metal must combine with a carrier molecule (e.g., protein) for transfer through the lipid bilayer. The protein binding can be specific or non-specific. Once the metal is through the membrane, it can then be released freely or exchanged with other intracellular carrier proteins. Once inside the cell, exchange to stronger ligands may occur, and the metal may be trapped inside the cell (Williams, 1981).

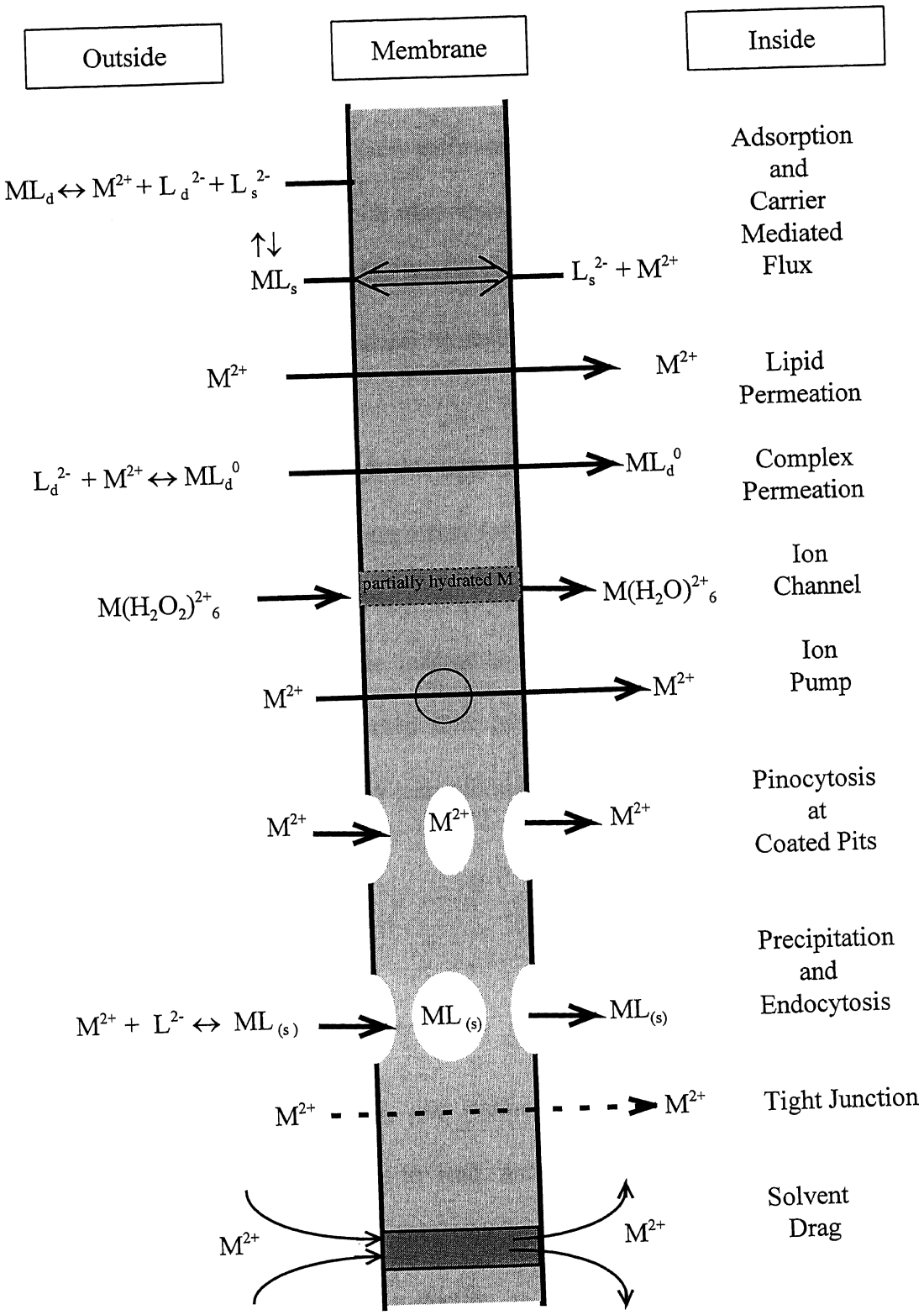


Figure 5. Possible mechanisms of ligand influence of metal (M^{2+}) passage across membranes (modified from Hamelink *et al.*, 1994).

Coupled associations of Pb with other elements may affect lead absorption. For example, if the body is deficient in either Fe or Ca, the bioavailability of Pb may be increased (Xintaras, 1992). Slow diffusion of lead to the small intestine and lead transfer across the epithelial cells may also be associated with phosphate bonding (Ragan, 1983). A covalent bond of lead to tissues in the form of lead phosphates has been suggested because the mucosal epithelial cells are naturally rich in phosphate ions (Blair *et al.*, 1979).

Simple diffusion through the tight junctions between absorptive cells into intercellular space can also be important for lead (Mushak, 1991). These junctions are negatively charged so they have a high affinity for the lead cation. Overall, the intestinal wall is a fluid mosaic infiltrated with protein and/or lipids. Due to its hydrophobic character, unrestricted flow of ionic species (i.e., most metals) from either the inner (luminal) or outer (serosal) sides does not occur.

2.6. Children

Infants, young children, and women of child-bearing age are the most vulnerable populations exposed to lead, and thus are the focus of the EPA's risk assessment efforts (Diamond, 1990). Typically PbB in a child increases from 0 - 2 years old, followed by a steady decline as the child grows older (Johnson *et al.*, 1996). Some reasons for the high vulnerability of infants and children are: sensitivity of developing organs (e.g., blood-brain barrier); behaviors that increase soil and dust

exposure; higher GI absorption rates than adults; and Fe and Ca deficiencies which may facilitate Pb absorption (Diamond, 1990).

Virtually all children in industrialized nations are chronically exposed to lead (Bellinger and Needleman, 1992). In 1988, an estimated 3 million children had blood lead levels > 15 $\mu g\ dl^{-1}$, which is above the recommended limit for PbB (Xintaras, 1992). These statistics are not meant to trivialize the importance of the detrimental effects of lead in adults, however it is also known that children absorb 3 - 10 times more lead than adults (Table 3) (Mahaffey, 1977; WHO, 1995). If an adult and a child intake the same amount of lead, the young child may absorb up to 50% of the lead, while the adult may only absorb 10 - 15% of the ingested lead.

Table 3. Comparison of daily lead intake and excretion in children and adults (WHO, 1995).

Parameter	Children	Adults
Dietary intake ($\mu g\ kg^{-1}$)	10.76	3.63
Fraction absorbed	0.55	0.15
Dietary lead absorbed ($\mu g\ kg^{-1}$)	5.92	0.54
Air lead absorbed ($\mu g\ kg^{-1}$)	0.20	0.21
Total absorbed lead ($\mu g\ kg^{-1}$)	6.12	0.75
Urinary lead excreted ($\mu g\ kg^{-1}$)	1.00	0.47
Endogenous fecal lead ($\mu g\ kg^{-1}$)	1.56	0.24
Total lead excreted ($\mu g\ kg^{-1}$)	2.56	0.71
Excreted/absorbed	0.42	0.92
Fraction of intake retained	0.33	0.01

One of the primary paths of lead ingestion is hand-to-mouth behavior, and children are much more prone to this than adults. Small particles can adhere to a child's hands during regular play (Day *et al.*, 1979). Furthermore, there is an inverse

relationship between soil particle size and the resulting amount of material adhering to the hands (Duggan *et al.*, 1985). Once these particles adhere to the hands, they can be ingested and thus become bioavailable. Temperature, humidity, and soil moisture content may also play a role in whether particles will adhere to a child's hands.

Children who recover from Pb poisoning have been shown to have short attention spans (Bellinger and Needleman, 1992), and evidence is building in support of lead's detrimental effects on IQ. In fact, Pb may affect development even after exposure ceases (Luthman *et al.*, 1994). Both experimental and epidemiological studies show behavior deficits in Pb exposed children (Rice, 1996). Researchers such as Needleman and Gatsonis (1990), Bellinger and Needleman (1992), and Schwartz (1994a) have examined the causal effects of Pb and IQ by using statistical analysis (Figure 6). Though the link between lead and IQ impairment has been established, direct causation is more difficult to prove (Needleman and Gatsonis, 1990).

In connection with the vulnerable child model, pregnant women are also listed in the high risk category, both for their own safety, as well as the safety of the fetus (Mahaffey, 1977). Between the mother and the fetus, transplacental transfer and subsequent buildup of lead can be a hazard because the placenta does not act as a barrier to filter the mother's blood lead concentrations (Barltrop,1969; Rabinowitz *et al.*, 1984; Choudhury *et al.*, 1992; Needleman, 1992; Hamilton *et al.*, 1994; WHO, 1995).

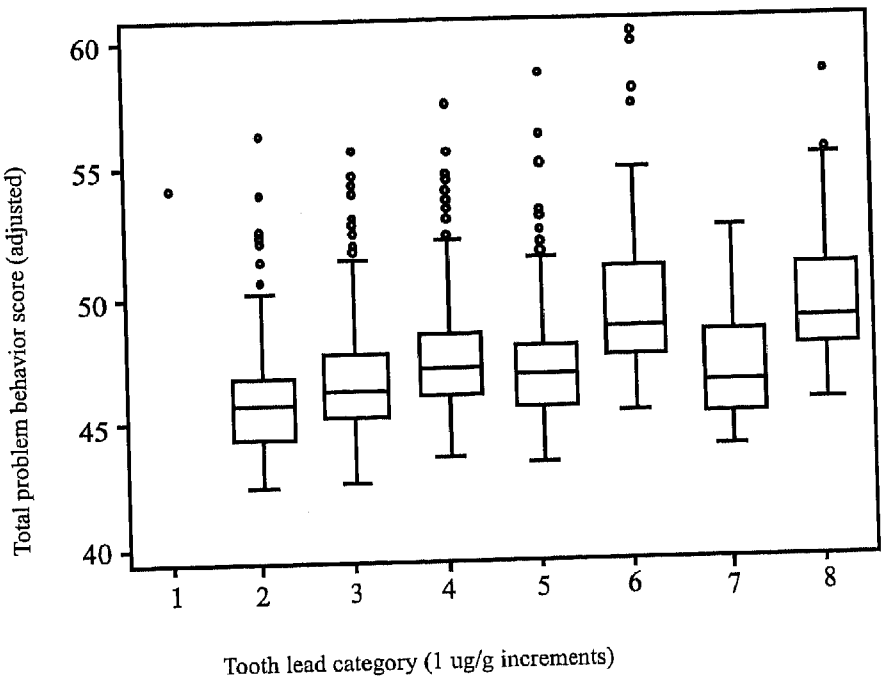
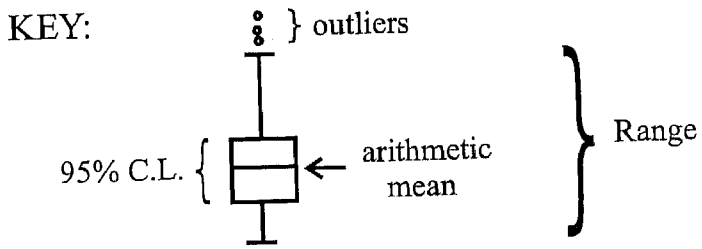


Figure 6. Box plot of "problem behavior" scores of children classified by tooth lead level (Bellinger et al., 1994).



III. MATERIALS & METHODS

In this study, a simple two stage *in vitro* digestion procedure is used to mimic absorption of lead from the GI tract: Stage I, the stomach phase; and Stage II, the intestinal phase (Table 4).

Table 4. Summary of *in vitro* experimental procedure.

Step	Operation
Stomach Phase:	
1	Weigh out 2.25 g of representative, dried & sieved (< 250 μm) test sample.
2	Add 250 mL stomach solution (Table 5) & weighed test sample to reaction vessel.
3	Place vessel in heated water bath (37°C).
4	Turn on argon gas over exposed surface.
5	After 10 minutes, turn on stirring rod.
6	Collect and filter (< 0.45 μm) 5 mL samples with disposable syringe at 20, 40, & 60 minutes after start of experiment; (replace removed volume with 5 mL stomach solution).
Intestinal Phase:	
7	Add two dialysis bags filled with 5 g $NaHCO_3$ and DI H_2O to reaction vessel.
8	Collect 5 mL samples at 30, 60, and 120 minutes after start of intestinal phase.
9	At pH 6.5 remove dialysis bags and add 0.4375 g porcine bile extract, and 0.125 g pancreatin directly to reaction vessel.
10	Maintain pH of 7 for remainder of experiment.
Total run time = 3 h	

To the medical community, "*in vitro*" deals with taking an organ from an animal and testing it outside the body (see Hazell and Johnson, 1987; Diepenmaat-Wolters and Schreuder, 1993; Minihane *et al.*, 1993 Wolters *et al.*, 1993; Shen *et al.*, 1994). In the experimental procedure listed in this report, the physiology of the stomach and intestines are simulated with synthetic components.

This *in vitro* procedure is modified from a method by Ruby *et al.* (1993), and is designed around the gastrointestinal tract of a 2 - 3 year old child. Ruby *et al.* (1993) originally adapted their design from an assay method for bioavailable Fe in food, by Miller *et al.* (1981). Following is a detailed description of the ten steps outlined in Table 4. Also following is a description of the three major components of the human digestive system: (1) the mouth, throat, and esophagus; (2) the stomach; and (3) the intestines (Figure 3).

3.1. Phase I - Stomach Phase

Step 1 - soil sample

A representative site sample should be chosen if only one sample is to be run; otherwise a larger array of samples should be run to provide a statistically meaningful *in vitro* bioavailability factor (BAF) for a particular site. Metal content by XRF (X-ray fluorescence; LEGS Standard Operating Procedure, 1993) of each pre-*in vitro* solid sample must be known to calculate the BAF after the experiment is done.

Weigh out a 2.25 ± 0.01 g split of dried and sieved (< 250 μm , Nalgene sieve) sample. This size range was used in the corresponding EPA *in vivo* study to represent the size fraction that may adhere to a child's hands, clothes, toys, etc. This particle size (< 250 μm) is also used in this *in vitro* experiment. In the future, the test particle size fraction may need to be raised or lowered (Duggan *et al.*, 1985; Kissel *et al.*, 1996). For example, the > 150 μm size fraction may adhere to hands when moisture content exceeds 10%; however if soil is dry, then < 65 μm seem to adhere the strongest (Kissel *et al.*, 1996). Other factors may also play a role in evaluating the adhesion of a particular soil to the skin, such as relative humidity, soil temperature, organic matter, clay and mineral content, and single soil exposure vs. continuous exposure. An equation describing the "concentration enrichment" due to increased surface area of smaller particles might be applicable to some exposure situations (Sheppard, 1995).

Step 2 - reaction vessel

The "stomach" is a specially designed polypropylene cylinder (Figure 7). Each cylinder holds 250 mL of simulated gastric juice (Table 5) and 2.25 g of contaminated test sample. The stomach (Figure 8) acts as a reservoir to receive all the food at once, while delivering it to the intestine in intervals; though gastric emptying can occur rapidly depending on the physiological state of the ileum.

In the reaction vessel, combine 2.25 g test sample and 250 mL stomach solution. This ratio of *2.25g: 250 mL* has been arbitrarily chosen. Research has

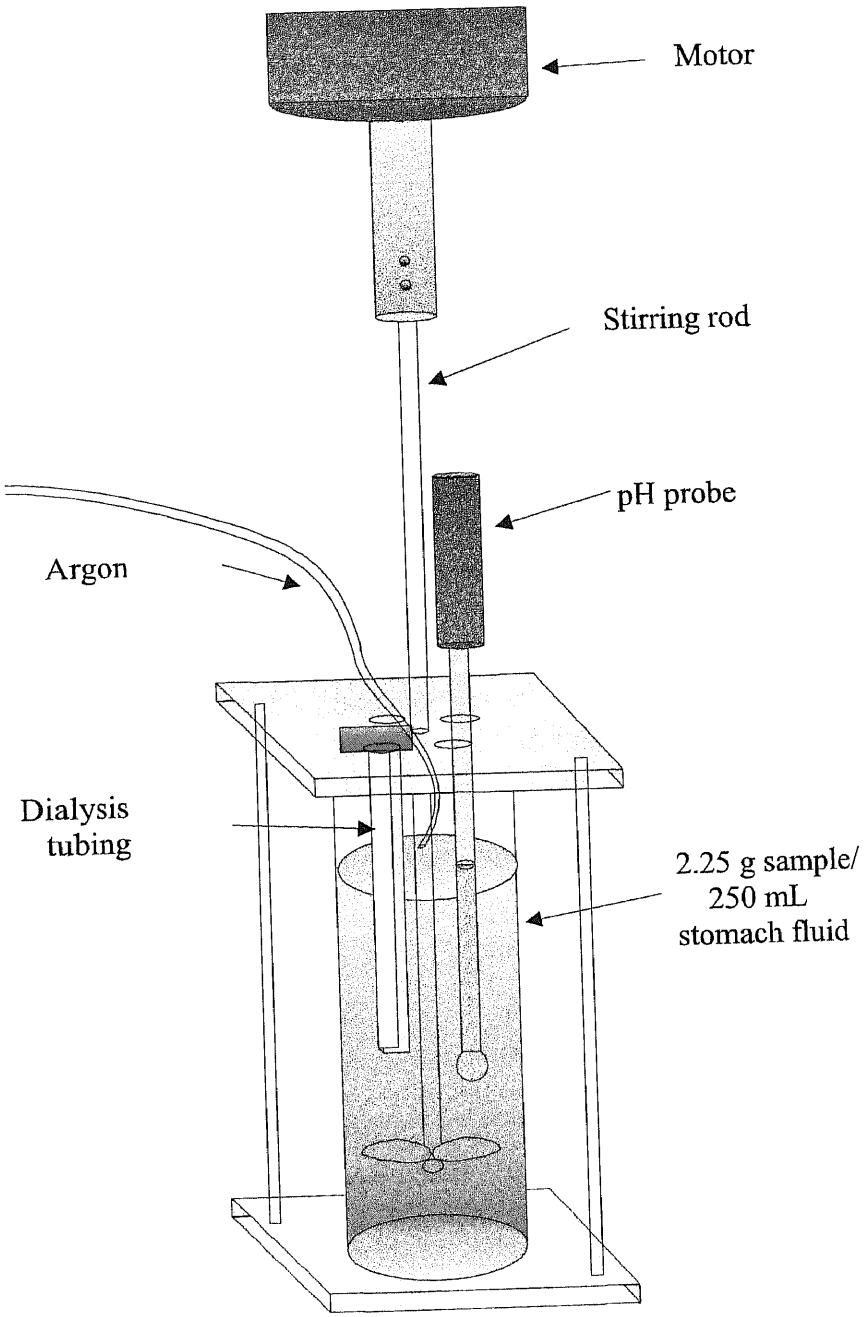


Figure 7. Diagram of in vitro reaction flask.

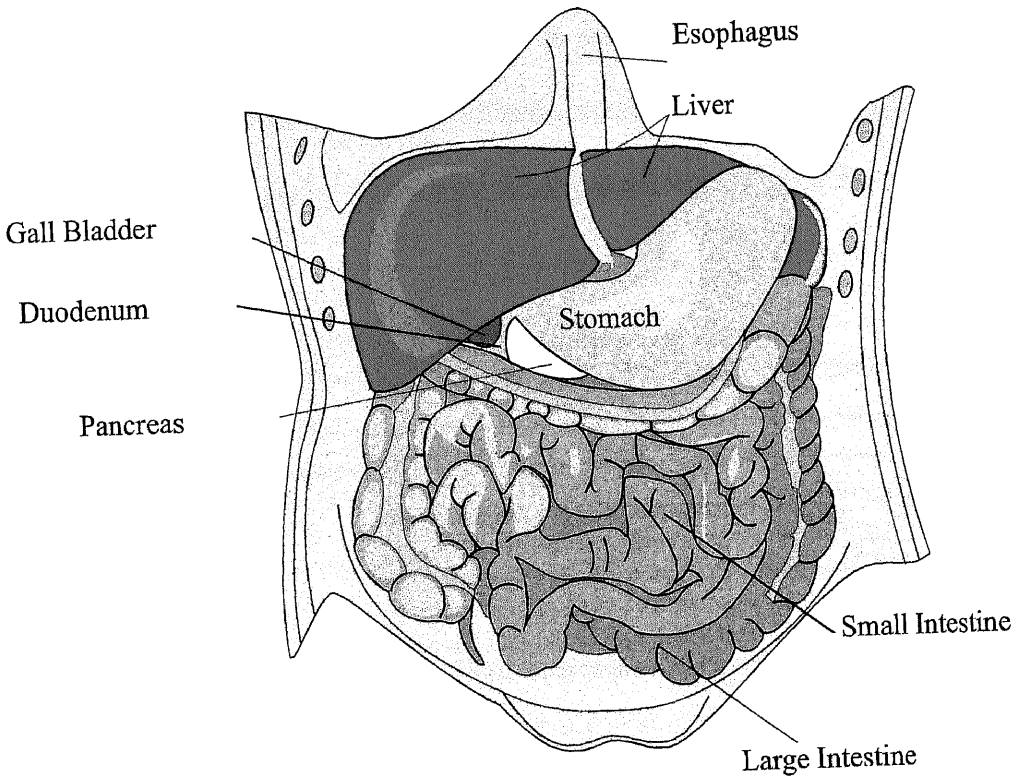


Figure 8. Diagram of human stomach and intestines.

shown that an average 2 - 3 year old child may ingest 80 - 200 mg soil day^{-1} (Day, 1979). However, as little as 1 - 2 mg day^{-1} for 5 - 6 months could cause lead poisoning in a 1 - 2 year old (Mahaffey, 1977).

The average stomach can hold approximately 1 L of fluid; whereas the entire digestive tract can secrete and absorb up to 10 L of fluid per day. The *in vitro* "stomach" holds 0.25 L fluid. The synthetic stomach solution is prepared by adding pepsin and various stomach acids to 4 L of deionized water (Table 5).

Table 5. Components of *in vitro* simulated stomach solution.
(Note: all chemicals from Sigma Chemical Co., St. Louis, MO, unless otherwise noted).

Amount	Compound
5 g	pepsin A (from porcine stomach mucosa; EC 3.4.23.1)
2 g	citric acid anhydrous (Fisher Sci, NJ; USP $C_6H_8O_7$; FL-03-0791)
2 g	DL -malic acid (DL - hydroxybutanedioic acid; $C_4H_6O_5$; 617-48-1)
1.68 mL	DL -lactic acid ($C_3H_6O_3$, synthetic: 85% (w/w) syrup approx.98%)
2 mL	acetic acid glacial (Amer. Sci. Prod., IL; CH_3COOH; UN2789)
variable*	hydrochloric Acid (trace metal grade)

* to bring to desired pH

The human stomach secretes hydrochloric acid (HCl), by parietal cells in the gastric epithelial mucosa, and the enzymes: pepsin by chief cells, renin, and lipase which help digest carbohydrates, proteins and fats. The process of chemical digestion is mainly accomplished by the HCl and various enzymes. The larger, more complex molecules are gradually broken down to smaller components that can be absorbed and delivered to the body's cells for consumption. Other acids that have been incorporated into the *in vitro* stomach fluid are: malic acid, citric acid, lactic acid, and

acetic acid. Citric acid has been shown to increase the bioavailability of lead (Wolters *et al.*, 1993).

Hydrochloric acid is the most important acid in the stomach that acts to solubilize metals. The parietal cells produce an excess of hydrogen ions (H^+) in the gastric juice, 10^6 times that in the plasma outside the stomach. This process is catalyzed by carbonic anhydrase, according to the reaction:

$$CO_2 + H_2O \rightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

As a result of this reaction, the bicarbonate anion (HCO_3^-) diffuses into the plasma while H^+ is actively "pumped" together with Cl^- into the stomach to produce hydrochloric acid.

The average pH of the stomach can range from as low as 1 to as high as 6, depending on food content and physical health of the subject (Tovar *et al.*, 1993). The pH of the stomach is an important parameter for bioavailability assessment. The usual pH of a fasting child is around 1 - 1.5; a higher pH, more typical of a stomach with food in it, is expected to lower the resulting bioavailability of ingested lead because the solubility of Pb decreases with increasing pH. The pH of the *in vitro* stomach solution is continuously monitored throughout the duration of the experiment; a few drops of HCl can be added if necessary to keep the pH down (especially for "slag" samples which tend to increase in pH when added to stomach solution).

Step 3 - heat

The solution is heated in a water bath (Figure 9) to 37°C (~98.6°F) before the sample amount is added. The reaction vessel remains in this heated environment for the entire run of the experiment. This parameter mimics that of normal internal body temperature.

Step 4 - argon

Turn on argon gas (or any inert gas) over the exposed surface of the reaction vessel to avoid any unnatural or excessive formation of oxidation complexes during the experiment.

Step 5 - stirring rod

The time the sample stays in the mouth, throat, and esophagus (Figure 3) is equated to the 10 minutes before the stirring rod is turned on in the experiment. In the mouth, salivary glands secret a mucus that aids in mechanical digestion and dissolving of food. The throat and esophagus transport these contents into the stomach.

The stirring rate of this particular *in vitro* experiment is 60 rpm (Arrow 1750 motor; Arrow Engineering Co., Inc., Hillside, N.J.). The stirring rate needs to be vigorous enough to keep particles in suspension, but not too vigorous as to overestimate the mechanical digestion process in the digestive tract. In mechanical

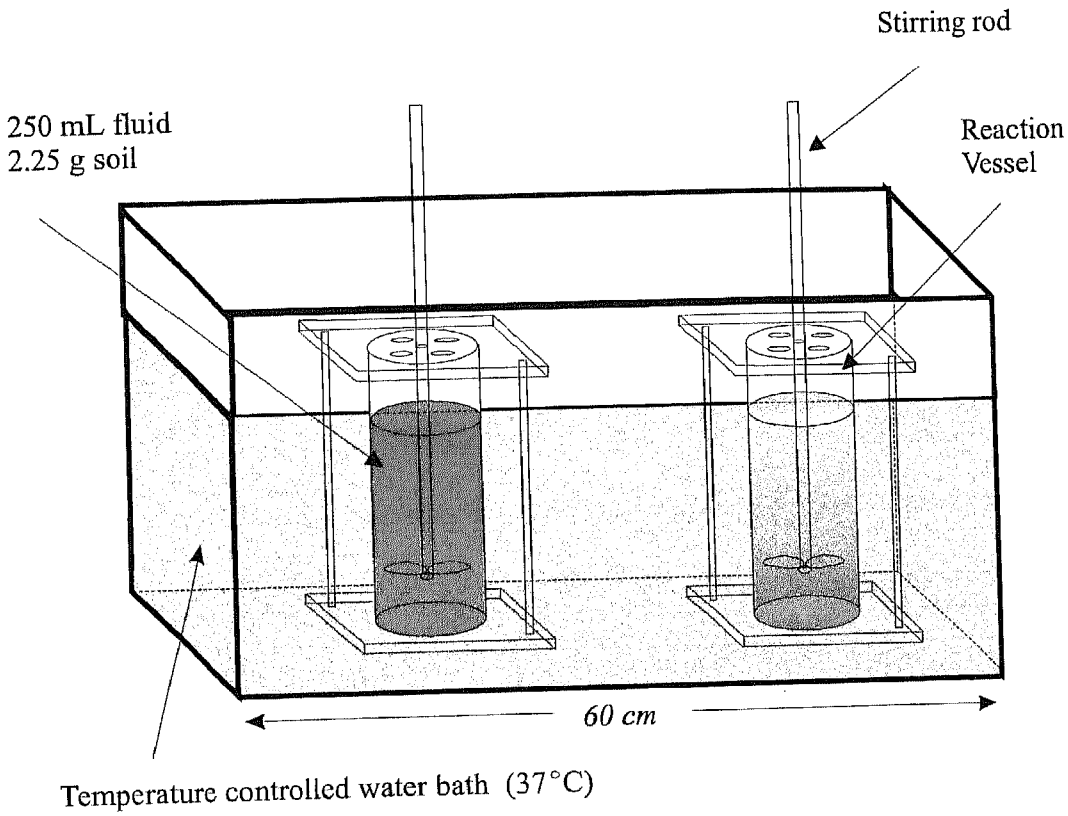


Figure 9. Diagram of in vitro test system.

digestion, solid food mixes with various juices from the digestive glands to dissolve the food as much as possible before chemical digestion occurs.

Step 6 - sampling events

Collect and filter 5 mL samples at 20, 40, and 60 minutes after the start of experiment. Temporarily turn off the stirring rod to collect samples. Replace the volume of solution removed at each sampling with 5 mL of stomach solution to maintain a constant volume in the remaining experiment. Collect samples with disposable 5cc sterile syringes (Becton Dickinson; Franklin Lakes, New Jersey), filter with disposable 0.45 μm cellulose acetate filters (Microfiltration Systems; Dublin, CA), and store in disposable 10 mL centrifuge tubes. The pipettes, filters, and storage tubes are used only once to minimize any contamination from recycled labware. Analyze samples for metals on an ICP-AES (Inductively Coupled Plasma- Argon Emission Spectrometer, ARL Model; Fisons; LEGS Standard Operating Procedure, 1993).

Gastric emptying time can vary from minutes to hours. In this *in vitro* experiment the stomach phase runs for 1 hour. Time is a crucial factor for metal solubility in transit (VanDokkum, 1989). Bioavailability of a metal depends in part on the species (complexed or uncomplexed) formed in the GI tract (VanDokkum, 1989). It has been suggested that galena (PbS) may form $PbCl_2$ in the presence of HCl in the stomach fluid (Healy *et al.*, 1982). If this is the case, the bioavailability of $PbCl_2$ would dominate over PbS in the stomach system, however *in vitro* results

suggest this transformation does not occur (Medlin and Drexler, 1995). However, even if the metal species is capable of undergoing chemical alteration, it may not have enough time to be fully altered if the contents are emptied out quickly.

3.2. Phase II - Intestinal Phase

Step 7 - $NaHCO_3$ -filled dialysis bags

After a 1 hour stomach phase, the "intestinal phase" begins. Two lengths of dialysis tubing (non-recycled, wet in 0.1% sodium azide, cellulose ester molecular-porous membrane, 100,000 MWCO, 12 mm flat width, 7.5 mm diameter; Spectra/Por, Houston, TX) filled with 5 g sodium bicarbonate (ACS Reagent 144-55-8, $NaHCO_3$; Sigma Chemical Co., St. Louis, MO) and deionized water (DI) are added to the heated reaction vessel. Direct addition of $NaHCO_3$ to the solution could create "micro environments" of high pH, resulting in the precipitation of metal compounds. This osmotic membrane also acts to simulate the gradual transfer of the sample from the small intestine to the bloodstream. Other *in vitro* techniques utilize actual intestinal organs to test for bioavailability; a possible drawback to using a natural intestine is that it is very thick, and transport is unaided by blood when the organ is removed from the body (Payne, 1989).

As the contents from the stomach reach the intestines, digestion continues in an alkaline environment (Figure 8). Lining the small intestine are absorptive cells forming a monocellular epithelium, covering the villi. Uptake in the intestine is

controlled in part by the enterocytes associated with the microvilli in the lumen (Mushak, 1991). In humans, the lumen of the small intestine has a gross cylindrical surface area of about 40 cm^2, but the actual surface area includes all the microscopic folds, and results in an enormous surface area of 200-300 m^2!

In the small intestine, the dissolved contents from the stomach are subjected to further mechanical mixing with the intestinal juice and movements that propel them forward. The first section of the small intestine, the duodenum, is about 20 cm long. It accepts the semi-digested contents from the stomach. The duodenum secretes the enzymes invertase and erepsin that are necessary for digestion. According to researchers, the primary area for Pb absorption is in the duodenum where Pb enters the epithelial mucosal cells (Conrad and Barton, 1978; Ragan, 1983).

The intermediate section of the small intestine, the jejunum, measures about 2 m in length. The jejunum swiftly carries digested food through the small intestine, therefore it seldom has much food in it. The last, and longest segment of the small intestine, the ileum, is about 4 m long. The brush border (microvilli plus glycocalyx) contains digestive enzymes to carry out the final stages of digestion in the absorptive cell membrane.

The large intestine, also known as the colon, accumulates waste products from digestion, dehydrates, and prepares them for excretion. The last function of the digestive system is to eliminate the unused waste materials without interfering with the processing of the incoming food.

Step 8 - sampling events

Obtain a 5 mL sample directly from the solution in the reaction vessel at 30 minutes after start of intestinal phase. Filter, acidify (nitric acid, Optima HNO_3; Fisher Scientific, Fairlawn, NJ), and analyze the sample for metals (ICP-AES). Replace the removed volume (5 mL) with deionized water to avoid any concentration error.

Step 9 - bile and pancreatin

Allow the pH of the reaction vessel fluid to reach 6.5 (approximately 30 minutes, depending on initial pH of sample) before removing the dialysis bags. At the neutralization point, add 0.4375 g bile salts (bile extract - porcine, 8008-63-7; Sigma Chemical Co., St. Louis, MO) and 0.125 g pancreatin (from porcine pancreas, 8049-47-6; Sigma Chemical Co., St. Louis, MO) directly to the reaction vessel.

The fluid of the small intestine is generally a bicarbonate-rich medium that acts to neutralize the acidic contents delivered from the stomach. The intestine contains secretions from the glands of the small intestine and from other glands such as the pancreas (pancreatin) and the liver (bile). In terms of digestion, bile salt from the liver acts as a detergent to emulsify fat droplets so lipases can act on them. The gall bladder, liver, and pancreas deposit their enzymes and bile into the duodenum.

Step 10 - Conclusion

Maintain pH of approximately 7 for the remainder of the experiment. The pH should continue to rise approximately 0.5 pH points after the $NaHCO_3$-filled dialysis

bags have been removed from the solution. Take two final samples at 60 and 120 minutes after the start of the intestinal phase. The total run time of experiment is 3 hours.

QA/QC (quality assurance/quality control) consists of 10% of samples run as duplicate or spike through the *in vitro* experiment, as well as a spike, duplicate, blank, and standard every 10% of samples analyzed on the ICP-AES.

3.3. Calculation of Bioavailability Factor (BAF)

The amount of Pb^{2+} found in solution from sampling the stomach phase is used to calculate the percent bioavailability (% BAF) of lead. Typically, it is the small intestine that absorbs lead across the human GI tract; however some fraction of this Pb^{2+} is likely to be excreted via the bile duct before entering the systemic circulation (Figure 2).

The following equation is used to calculate the bioavailability factor (% BAF):

$$\%BAF = \left\{ (L)(M_{aq}) \Big/ \frac{(S)(M_s)}{1000} \right\} * 100$$

L = volume of stomach solution : 0.25 L (constant)
(density of stomach solution is 1);
M_{aq} = amount of Pb^{2+} in stomach solution samples [mg kg^{-1}];
(measured by ICP-AES)
S = weight of initial soil sample: 2.25 g (constant);
M_s = amount of metal in initial bulk soil sample [mg kg^{-1}];
(measured by XRF).

The % BAF is not a measure of the *absolute* bioavailability of lead in a system; it is a measure of *relative* bioavailability. However, this relative % BAF can be used to calculate the absolute bioavailability of a metal phase in any biological system. The % BAF calculated directly from the *in vitro* experiment can be thought of as 100% lead absorption. The absolute bioavailability for a particular human system will always be less than this 100% absorption. For instance, adults are thought to absorb 5 - 15% of the lead ingested; to calculate the absolute BAF from a lead-dosed sample, the "relative BAF" is multiplied by a factor of 0.05 to 0.15. For a 5-year-old child, the relative BAF is multiplied by 25% ; and for an infant the multiplying factor is 50% (Casteel *et al.*, 1996; Mushak, 1996). These coefficients represent the expected absorption amounts of lead from the gastrointestinal tract (default values for lead absorption in the EPA's blood Pb estimation model, IEUBK, are: 50% for dissolved Pb, and 30% for Pb in soil) (Casteel *et al.*, 1996). In general, infants may absorb 5 times more lead than adults, and 2.5 times more than older children.

3.4. Individual *In Vitro* Tasks

Task 1. Reproducibility of the In Vitro Method

To test the reproducibility of the *in vitro* method, a NIST standard, MS 2710, was run through the stomach phase six separate times. This sample, "Montana Soil

2710" (Trahey, 1995), is a very fine-grained, homogenous soil with 5532 mg kg^{-1} Pb ± 80 mg kg^{-1}; and 626 mg kg^{-1} As ± 38 mg kg^{-1}. In addition to testing reproducibility, the uncertainty of the *in vitro* method was examined by calculating : (1) the average relative percent difference [RPD = (a-x)/((a+x)/2)] of duplicate pairs (n = 98 pairs); (2) the average relative percent difference of spiked (spike : 20 mg kg^{-1} Pb) sample pairs (n = 113 pairs); and (3) the average relative percent difference of 79 "post" *in vitro* samples analyzed on the ICP-AES.

Task 2. Calibration of In Vitro to In Vivo

Calibration of the *in vitro* model was done by comparing "relative bioavailability *in vitro* results" to the "relative bioavailability *in vivo* results" for the same soil samples (though *in vivo* results are still preliminary). Comparison was done by simple linear regression between the two data sets.

In this case, the swine was chosen as the acceptable animal (*in vivo*) model. Relative percent bioavailability of lead and arsenic samples from the *in vivo* study was calculated by modeling both the "dose-response" or "dose vs. blood lead" concentration curves for soluble lead, and the "biological response" in a dosed soil-lead sample. A comparison between the reference (lead acetate) and the test material (dosed soil-lead sample) is made in order to find the ratio of *doses that produce equal biological responses* (not the ratio of responses at equal doses; Figure 10) –this is then used to calculate the relative percent bioavailability of a sample (Casteel *et al.*, 1996).

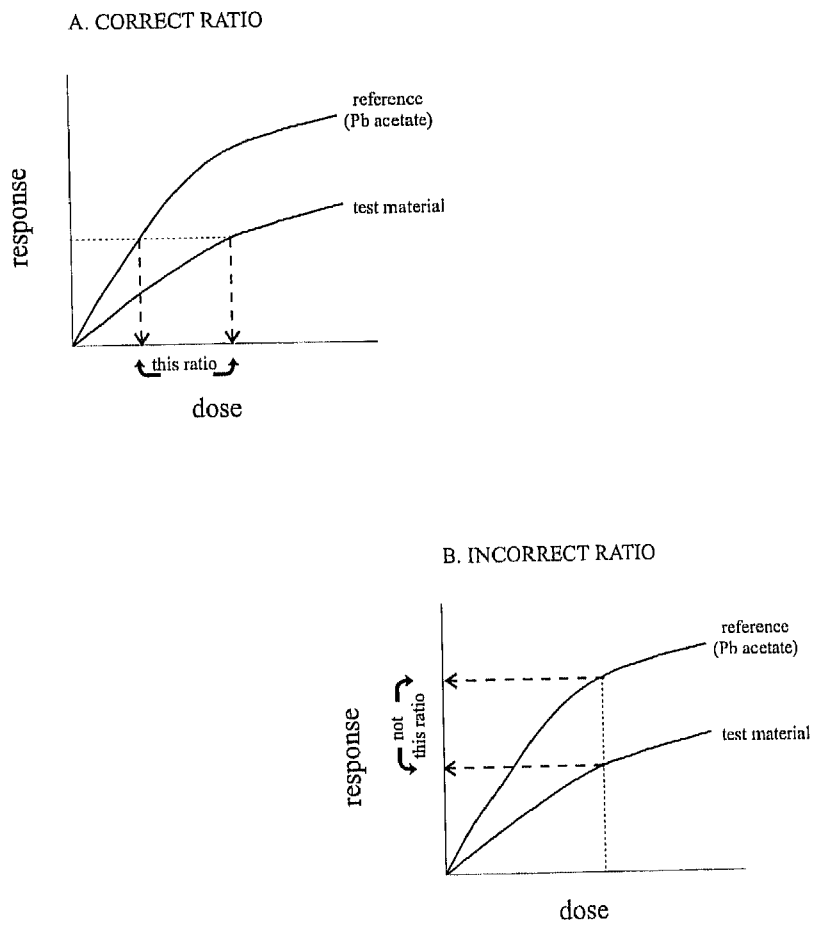


Figure 10. Graphical representation of in vivo relative bioavailability. (Brattin B., personal comm., 1997).

Samples from NPL/Superfund sites with high concentrations of heavy metals were chosen by the EPA to dose swine with Pb and As in an elaborate bioavailability study (Table 6) (Weis *et al.*, 1995 for swine study design).

Table 6. List of *draft* samples from EPA swine feeding studies; used for calibration of *in vitro* procedure. (*Note:* Most predominant Pb or As mineral is listed first in each phase category).

Site location	Name/Type	Primary Pb phase	Primary As phase
Bingham Creek, UT	hi Pb soil	$PbSO_4$/ Pb-P/ Pb-Fe-S	As-Fe-S
Bingham Creek, UT	low Pb soil	Pb-P/ Pb-Fe-S/ Pb-Mn-O	As-Fe-S/O
Jasper County, MO	hi Pb smelter	$PbCO_3$/ native Pb/ Pb-P	N/A
Jasper County, MO	low Pb yard	$PbCO_3$	N/A
Jasper County, MO	hi Pb mill	$PbCO_3$	N/A
Murray Smelter, UT	slag	Pb-As-O/ PbO	slag
Murray Smelter, UT	soil	Pb-As-O/ PbO	Pb-As-O
Aspen, CO	residential soil	$PbCO_3$	N/A
Midvale, UT	slag	Pb-As-O	slag
Leadville, CO	California Gulch	Pb-Fe-Mn-O	N/A
Leadville, CO	Arkansas Valley slag	Pb-As-O	slag
none	Pb soil + NIST paint	PbO	N/A
none	Pb soil + galena	PbS	N/A
Palmerton, NJ	location 2, soil	Pb-Mn-O	N/A
Palmerton, NJ	location 4, soil	Pb-Mn-O	N/A

KEY to Primary Pb & As phases:
$PbSO_4$: anglesite
Pb-P : lead-bearing phosphate
Pb-Fe-S : lead-bearing iron sulfate
Pb-As-O : $lead_x arsenic_{x-1}$ oxide
PbO : lead oxide
PbS : galena
Pb-Fe-Mn-O: Pb-bearing Fe & Mn oxides
Pb-Mn-O : lead-bearing Mn oxide
As-Fe-S : arsenic-bearing iron sulfate
$PbCO_3$: cerussite
As-Fe-O : arsenic-bearing iron oxide

Another sample used to calibrate the *in vitro* technique was from a human study conducted by Columbia University, New York, which consisted of a sample from Bunker Hill, Idaho (lead barite and Pb-Fe-sulfate-rich soil; sample names: Columbia 18 and Columbia 33). All samples used in the calibration of the *in vitro* method were run in triplicate.

Task 3. Dependence on Mineral Phase

Different inorganic Pb mineral phases were studied to assess whether the chemical form of lead had an impact on *in vitro* bioavailability. The lead samples used for this were: cerussite ($PbCO_3$); anglesite ($PbSO_4$); galena (PbS); pyromorphite ($Pb_5(PO_4)_3X$, where X = Cl, F, Br, or OH); lead oxide (PbO); slag; and Pb-bearing Fe and Mn oxides from natural cave deposits (Argo Mine, Serendipity, Cave of the Winds, Upper Pictograph, Hubbard, Groaning, Bida, Iron Geyser).

All samples, except for the Pb-bearing Fe and Mn oxides, were prepared by weighing a known amount of the lead sample (< 250 μm) and combining it with a known amount of washed silica sand. This process was used to keep the sample Pb content reasonably low (~ 5000 mg kg^{-1}) while still retaining the necessary 2.25 g dry weight needed for the *in vitro* procedure. For the Pb-bearing Fe and Mn oxides, the entire 2.25 g of sample was made up of the naturally occurring cave sediments (sieved to < 250 μm).

Task 4. Influence of pH on Bioavailability

The effects of stomach pH on the bioavailability of lead were studied by *in vitro*. An additional study was carried out in HCl/deionized water only (no other stomach acids were added) to compare with *in vitro* results. The pH values studied were: 1.3 and 3.0 (only in HCl), and the sample types were: synthetic $PbCO_3$; cerussite; synthetic $PbSO_4$; anglesite; native Pb; wulfenite ($PbMoO_4$); synthetic lead oxide; galena; and pyromorphite. In addition to those minerals that were studied for

dissolution in HCl only, four samples were run at varying pH's with the *in vitro* method (all the stomach acids added). These four samples were: galena, lead oxide, pyromorphite, and NIST MS 2710; the pH values compared by *in vitro* were 1.5 and 2.5. Samples were prepared by adding a known amount of Pb mineral to a known amount of washed silica sand.

Task 5. Fluid-to-Solid Ratio

The homogeneous NIST standard MS 2710 was tested for its lead and arsenic bioavailability response to a change in the ratio of *soil sample size to stomach solution* volume. The solution amount was kept constant (250 mL), while the soil amount varied : 0.5 g, 1.0 g, 1.5 g, 2.0 g, and 2.25 g. A pH of 1.5 was also kept constant in all tests.

Task 6. Particle Size Effects on Bioavailability

The effects of particle size were studied with 6 samples: anglesite, cerussite, galena, lead oxide, pyromorphite, and slag (a byproduct of the smelting process). Four particle size ranges were separated out of each sample type by wet sieving the entire sample through a series of stacked sieves: < 250 - 125μm; < 125 - 63 μm; < 63 - 38 μm; and < 38 μm. After wet sieving, samples were then oven dried (~ 90° C) and weighed. Final test samples were prepared by adding a precise amount of Pb mineral (± 0.001g) to a known amount of washed silica sand.

Task 7. HCl vs. Stomach Solution

Three samples were chosen to study the precise difference between the *in vitro* stomach solution (with its many acids), and a solution of HCl /deionized water. Each set of samples was run through the *in vitro* experiment for the same amount of time, and same pH, but different solution components. The solutions were compared by the absolute difference in their % bioavailability. These samples were MS 2710 (NIST), cerussite, and anglesite. Both the cerussite and the anglesite samples were prepared by adding a known amount of the Pb mineral to a known amount of washed silica sand.

Task 8. Intestinal Phase

Most of the samples presented in this thesis were run through the stomach phase only. However, a few samples were also run through the intestinal phase. Preliminary results are presented here. Samples run through the entire 3 hour experiment (stomach and intestine) were: MS 2710 (NIST) and lead oxide.

Task 9. Effects of Food on Bioavailability

The effect of food presence in the stomach on the bioavailability of lead was studied by *in vitro* method with three samples: lead oxide, pyromorphite, and galena. Preliminary data on a few foods (milk, banana, rice cereal, and oatmeal) are presented. The food was measured and added to the stomach solution to equal 250 mL. Three different ratios of *food : stomach solution* amounts were tested (10, 20, and 40 mL food added to total 250 mL). The pH of the samples was not adjusted

after the start of the experiment. The Pb test samples were prepared by adding a known amount of the Pb mineral (particle size : < 250 μm - 125 μm) to a known amount of washed silica sand.

IV. RESULTS

4.1. Reproducibility of *In Vitro* Method

The overall reproducibility of the *in vitro* method was examined for Pb from four different aspects: (1) sample duplicates; (2) sample spikes; (3) NIST standard: Montana Soil 2710; and (4) instrument (ICP-AES) QA/QC.

Of the 98 duplicate sample pairs examined (Figure 11) the average relative percent difference was 12.2%. The average relative percent difference of the spiked sample pairs (Figure 12) was even lower than the duplicates', with an average RPD of 6.1%. Both the duplicate and the spiked *in vitro* runs are within acceptable error for standardized testing.

The NIST MS 2710 (Figures 13 & 14) showed a standard deviation of only 1.5% for Pb, and 6.2% for As. This test standard (MS 2710) showed the procedure to be more accurate for Pb than As. The percent error for both elements (Pb and As) is well below the usual EPA recommended reproducibility limits.

The QA/QC of the ICP-AES produced an average relative percent difference of 6.0% (Figure 15). All four of these areas used to examine the reproducibility of the

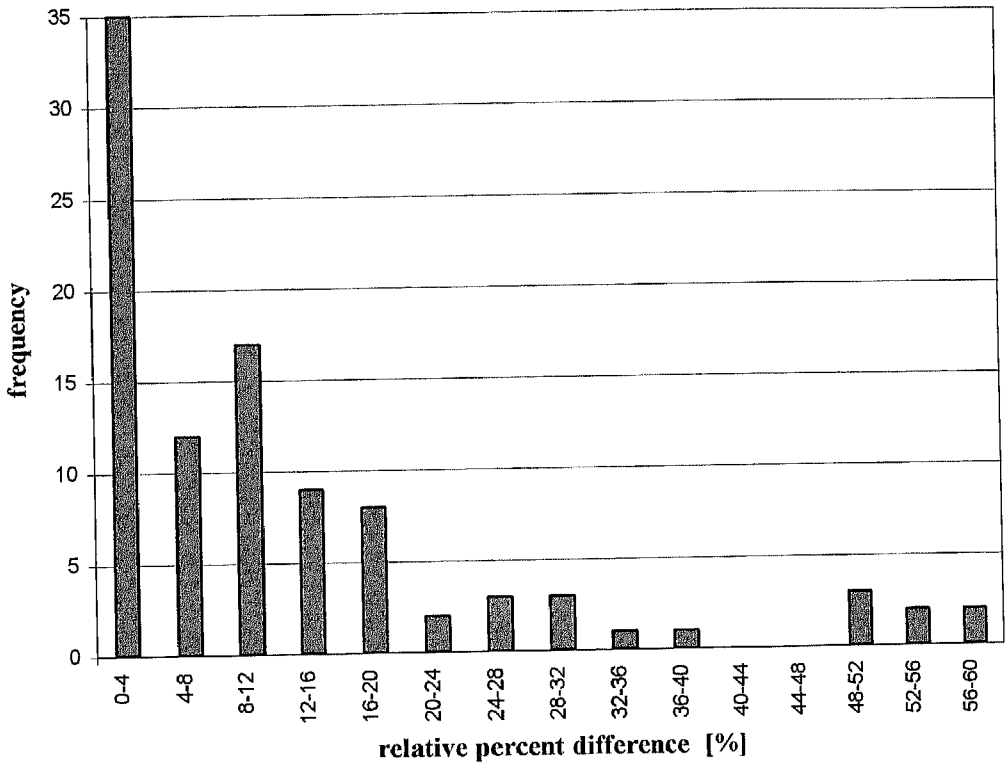


Figure 11. Frequency of RPD among in vitro duplicates.
[n = 98; average rpd = 12.2%]

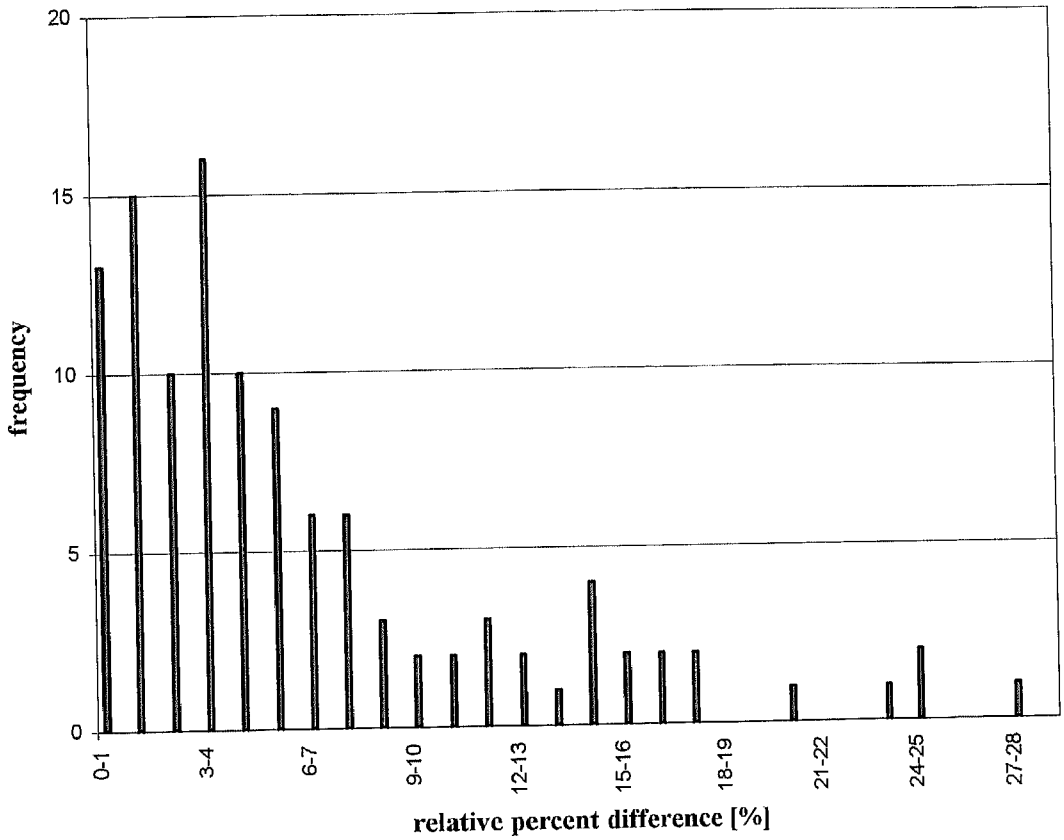


Figure 12. Frequency of RPD among in vitro spikes (Pb).
[n = 113; average rpd = 6.1%]

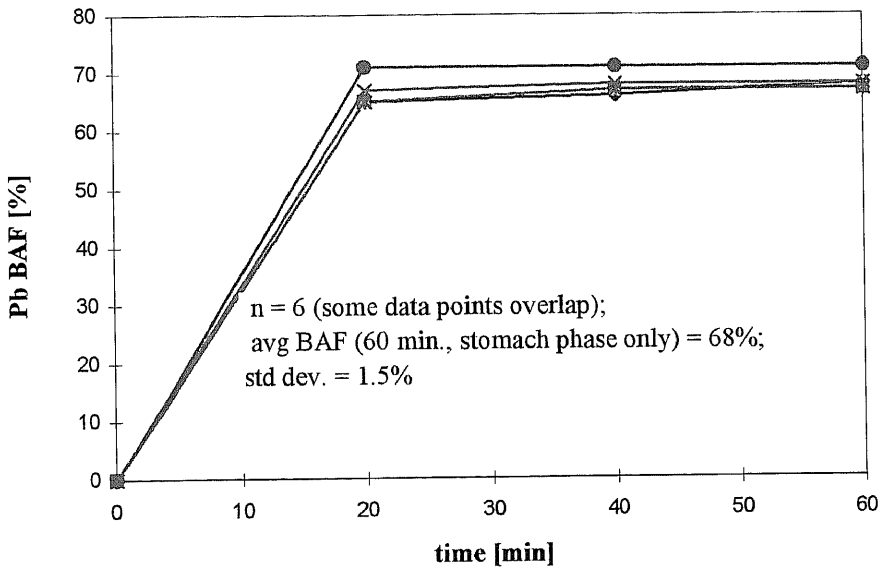


Figure 13. Precision of *in vitro* method.
Sample: NIST std MS 2710. Results for Pb only.

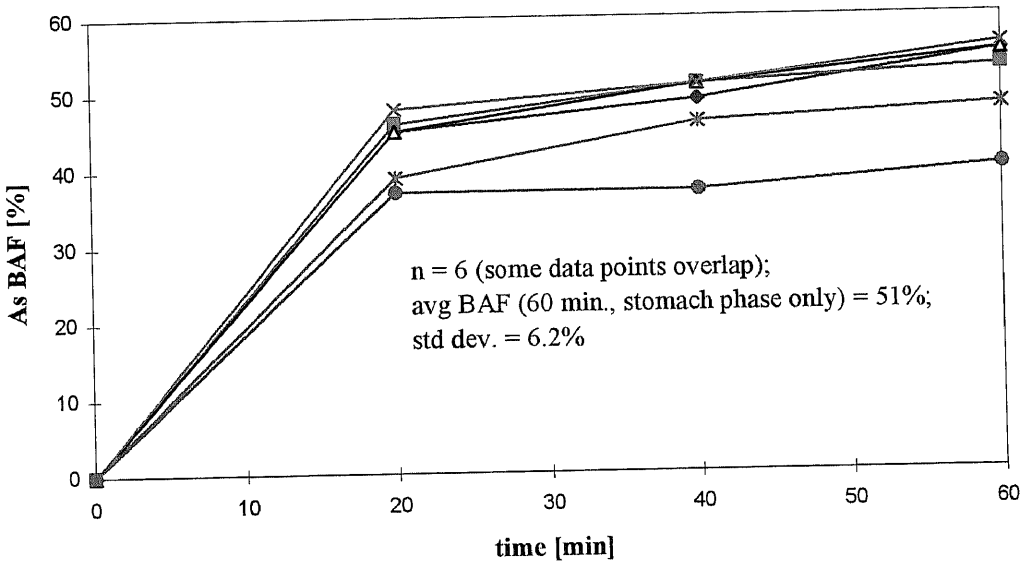


Figure 14. Precision of *in vitro* method.
Sample: NIST std MS 2710. Results for As only.

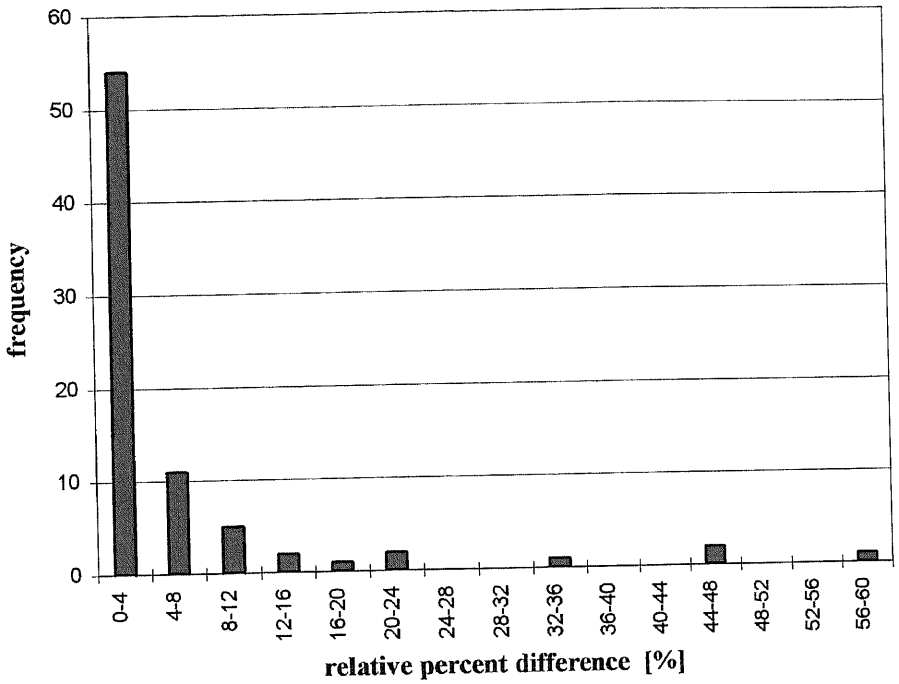


Figure 15. Histogram: Frequency of RPD among ICP dups, spikes, & stds. (QA/QC).
[n = 79; average rpd = 6.0%]

in vitro method fall within the acceptable range for good reproducible results. A conservative estimate of the expected percent bioavailability for a well-homogenized sample should remain within a ±10% margin of error when tested by the *in vitro* method.

4.2. Calibration of *In Vitro* to *In Vivo*

The most important aspect of this study was to establish a correlation between the BAF numbers from the EPA *in vivo* swine study, and the subsequent *in vitro* BAF numbers. To compare the *in vivo* to the *in vitro,* the same samples were used in both experiments (Table 7, and Appendix I).

Table 7. Results from *in vitro* and *in vivo* studies for Pb and As bioavailability

Site location	Name/Type	in vitro Pb	in vivo Pb*	in vitro As	in vivo As*
Bingham Creek, Utah	hi Pb soil	37	30	17	33
Bingham Creek, Utah	low Pb soil	47	32	38	n/a
Jasper County, Missouri	hi Pb smelter	65	56	59	n/a
Jasper County, Missouri	low Pb yard	73	78	0	n/a
Jasper County, Missouri	hi Pb mill	80	82	0	n/a
Murray Smelter, Utah	slag	66	55	60	56
Murray Smelter, Utah	soil	63	67	47	34
Aspen, Colorado	residential soil	76	58	N/A	N/A
Midvale, Utah	slag	14	20	18	24
Leadville, Colorado	Cal. Gulch	83	87	N/A	40
Leadville, Colorado	Ark.Valley	12	20	20	21
none	Pb soil + paint	67	82	N/A	N/A
none	Pb soil + PbS	1.6	1	N/A	N/A
Palmerton, New Jersey	location 2 soil	54	72	16	16
Palmerton, New Jersey	location 4 soil	56	58	32	32
Columbia	18 and 33	75 (RBA)	30 (ABA)	N/A	N/A

***Values are draft and subject to change with final evaluation by EPA.**

The two BAF components, when plotted on a Cartesian graph, give a curve that shows a direct relation between the two types of experiments (*in vivo* and *in vitro*). With the examination of EPA sample materials, the preliminary *in vivo* results were plotted against the *in vitro* results for both Pb and As using a simple linear regression model (Figures 16, 17 & 18). The resulting curves were surprisingly linear; a multiplier coefficient was expected to align the *in vitro* BAF to the *in vivo* BAF, but results showed they can be almost directly related on a one-to-one basis.

In the case of comparing the *in vivo* blood-lead bioavailability numbers with the *in vitro* bioavailability (Pb) numbers (Figure 16): m = 0.89, b = 5.5, and $r^2 = 0.85$ (and for comparing the "EPA suggested point estimate" (see Casteel *et al.*, p.27, 1996) to the *in vitro* (Figure 17), m = 0.87, $r^2 = 0.85$). The slope, being very close to 1 is a good indicator of a direct correlation. The r^2 being close to 1 is a good indicator of a close fit regression line. Likewise, comparing preliminary *in vivo* urinary-arsenic bioavailability numbers with the *in vitro* bioavailability (As) numbers (Figure 18): m = 1.5, b = -12, and $r^2 = 0.86$; arsenic *in vitro* bioavailability values are still preliminary however. The sample size for As was much smaller than the Pb sample size (As : n = 6; Pb : n = 15); more data points are needed to assess the predictability of the *in vitro* test for arsenic. The r^2 numbers best represent how close the two techniques are in mirroring one another; with respect to Pb, the *in vitro* technique is about 85% successful at predicting Pb bioavailability. In the case of As, the *in vitro* procedure is about 86% successful.

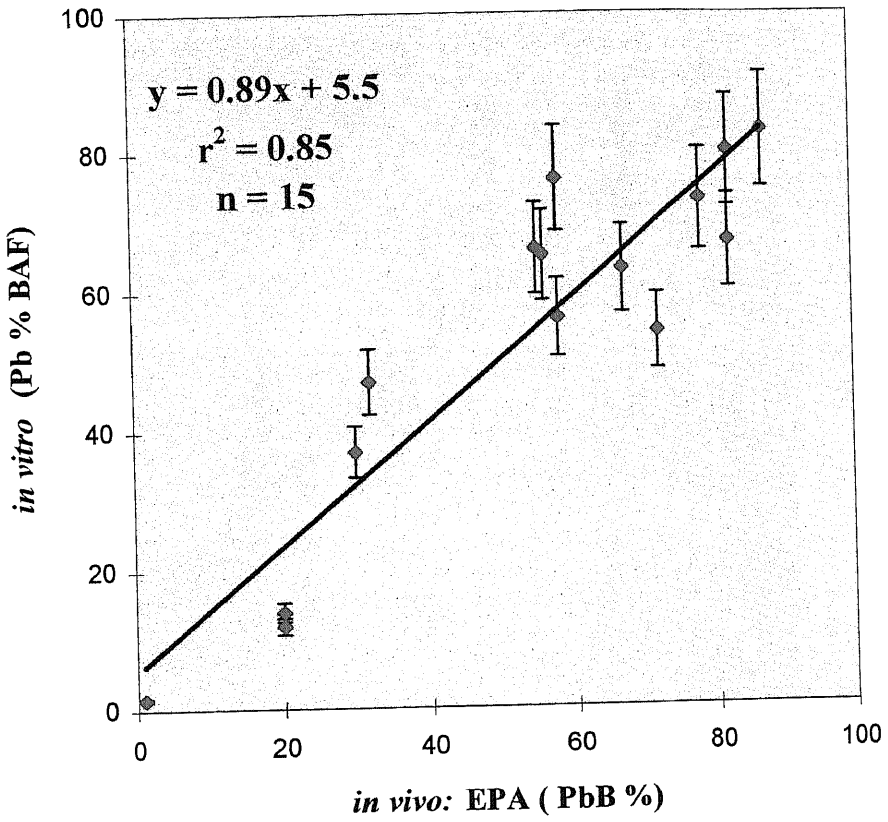


Figure 16. EPA swine blood lead vs. *in vitro* relative bioavailability.

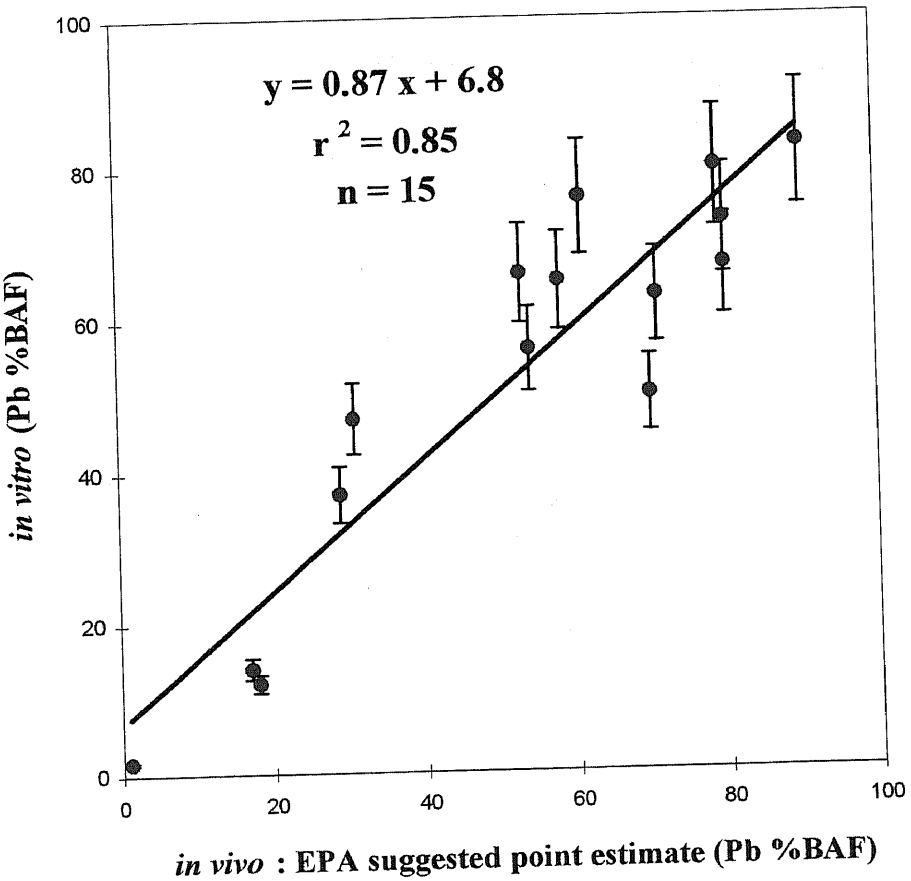


Figure 17. Lead results from calibration of *in vitro* to *in vivo*. EPA suggested point estimate for Pb, plotted against *in vitro* data for the same soil samples.

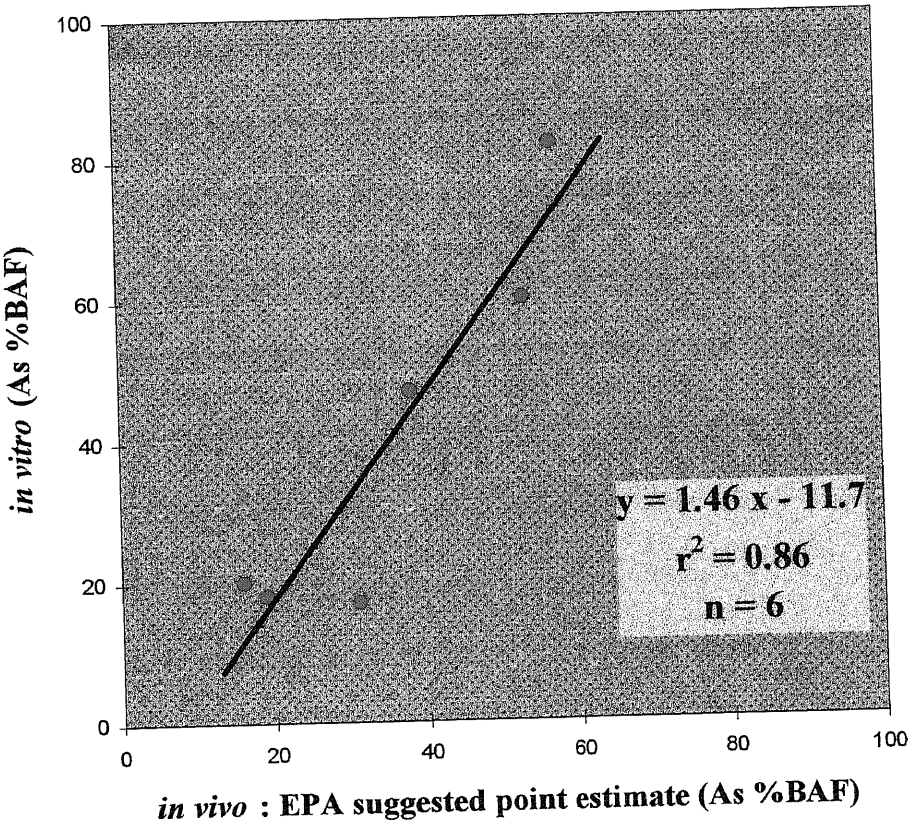


Figure 18. EPA swine study results for arsenic, plotted against *in vitro* data for the same soil samples.

4.3. Dependence on Mineral Phase

The solid mineral phase can play an important role in controlling the bioavailability of lead. *In vitro* bioavailability results (Figure 19) span the entire range, from 0% bioavailable to 100% bioavailable. According to the bioavailabilities predicted by the *in vitro* technique, lead phases with a relatively low bioavailability (< 50%) are: galena (K_{sp} PbS = 3.4×10^{-28} ; Mushak, 1991), pyromorphite (K_{sp} $Pb_5Cl(PO_4)_3 = 4 \times 10^{-85}$; Nriagu, 1973), and anglesite (K_{sp} $PbSO_4 = 1.82 \times 10^{-8}$; Lide, 1994). Also on the low end of the scale are lead-bearing Fe and Mn minerals, as well as lead-phosphates. However, the Pb-phosphates found in soil (i.e. Bingham sample) showed a much higher *in vitro* bioavailability than the pure crystalline form, pyromorphite. Lead phases with a relatively high bioavailability (≥ 50%) are: cerussite, and lead oxide, which are typically found in paints.

Slags can have either high or low bioavailability depending on the physical form of the small particles of Pb and As oxide included in the vitrified glass solid. A typical slag is a vitrified glass byproduct formed by the smelting process. Slags are chemically comprised of 33% of each: Fe_2O_3, CaO, and SiO_2, with trace Pb and As throughout. Slags may differ from one another by the degree of high lead particles that are partially or entirely free ("liberated") from the surrounding glass solid (Drexler, 1995). Some slags may have many free [$Pb_x As_{x-1} O$] particles that can be released when the glass matrix particle size is reduced, either by physical processes or by the acidic conditions in the stomach. This reduction in particle size can expose the

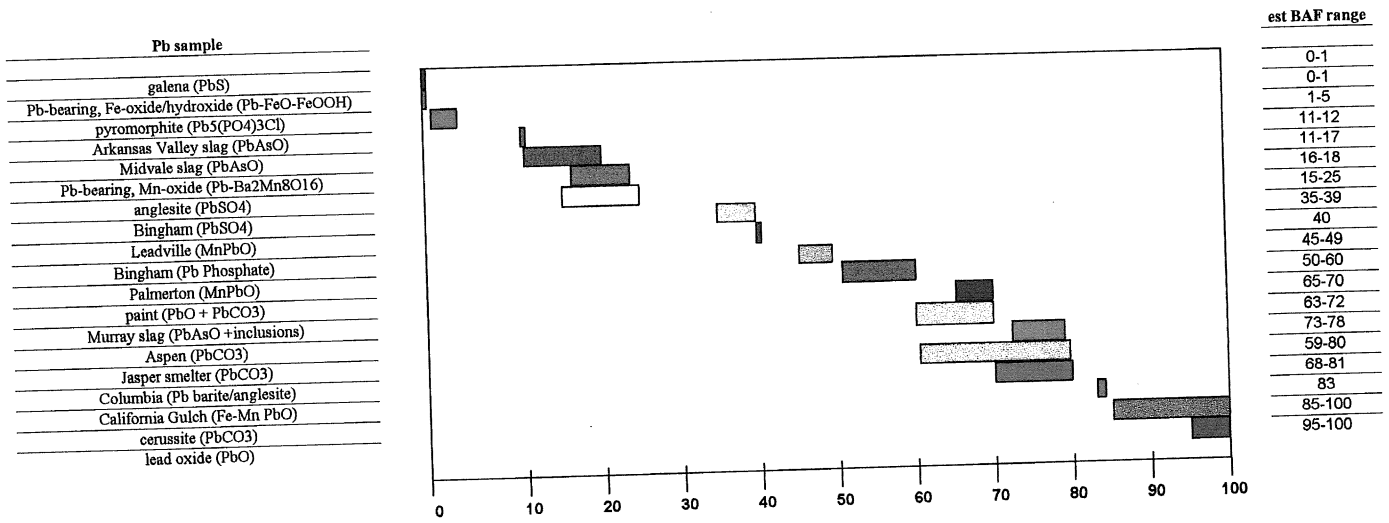


Figure 19. Mineral phase effects on the bioavailability of lead.

Pb and As particles to the stomach acid and potentially increase the bioavailability of Pb and As in a sample (e.g. Murray slag: 70% BAF, Figure 19). Other slags may have lead-arsenic particles that are encapsulated and protected from the acid of the stomach during digestion, thus resulting in a lower bioavailability (e.g. Arkansas Valley/Leadville slag: 10% BAF; and Midvale slag: 15% BAF; Figure 19). These slags are a good example of the fact that the physical form of the lead species may affect its bioavailability (Medlin and Drexler, 1995).

4.4. Influence of pH of Bioavailability

Changes in pH occur as material is passed along the gastrointestinal tract. As mentioned earlier, the pH of the fluid within a fasting child's stomach is typically around 1 - 1.5. In general, the solubility of heavy metal ions increases significantly when the pH is lowered below 5. Specifically, the change in Pb bioavailability was examined over a pH range of : 1.3 - 3.0 (Table 8). Results show the expected decrease in bioavailability with increase in pH. However, the small range of pH used and lack of data allows little insight into the sensitivity of the method for pH. Further tests with a wider range of pH (e.g. tested every 0.5 pH unit from 1 - 6) and more data points per sample is recommended.

The percent change in BAF was measured by comparing the change in bioavailability from the lower pH stomach to the higher pH stomach; any negative percent change would represent a lower bioavailability in the higher pH stomach. Of

the 9 different samples examined, all 9 were tested in a HCl/DI solution (Table 8, Experiment 2), and 4 were tested by the *in vitro* stomach solution as well (Table 8, Experiment 1). All samples were compared at two different pH's: low = 1.3 - 1.5, and high = 2.5 - 3.0. The dissolution of the Pb minerals in HCl/DI generally resulted in a lower BAF than expected with the *in vitro* experiment; the reason for this trend can possibly be explained be the absence of stirring in the HCl/DI experiment (samples in a 100 mL bottle, slowly agitated in a heated water bath).

Table 8. Effects of varying stomach pH on the bioavailability of lead.
Each sample run at two different pH's. (Experiment 1 = *in vitro*; Experiment 2 = HCl dissolution only).

Sample:	PbS	PbS	$Pb_5(PO_4)_3Cl$	$Pb_5(PO_4)_3Cl$	NIST MS 2710
Experiment #:	1	2	1	2	1
pH:	1.5 2.5	1.3 3.0	1.5 2.5	1.3 3.0	1.5 2.8
BAF:	0.1 0.2	0.1 0.2	1.4 0.5	1.6 0.0	67.3 60.3
% change in BAF (from low pH to high pH):	+100%	+100%	-64%	-100%	-10%

Sample:	$PbSO_4$	synthetic $PbSO_4$	Pb^0	$PbMoO_4$
Experiment #:	2	2	2	2
pH:	1.3 3.0	1.3 3.0	1.3 3.0	1.3 3.0
BAF:	0.6 0.5	0.8 0.6	0.3 0.5	0.3 0.1
% change in BAF (from low pH to high pH):	-17%	-25%	+67%	-67%

Sample:	PbO	PbO	Pb-Fe_2O_3	synthetic $PbCO_3$
Experiment #:	1	2	2	2
pH:	1.5 2.5	1.3 3.0	1.3 3.0	1.3 3.0
BAF:	94.1 62.4	15.0 3.4	0.2 0.0	12.0 4.5
% change in BAF (from low pH to high pH):	-34%	-77%	-100%	-63%

If the BAF's of the two different experiments ("HCl only" vs. *in vitro*) are compared on a relative sense only, the dissolution factors show the same trend in both experiments. Three of the samples were compared with both methods: PbS, $Pb_5(PO_4)_3X$, and PbO. Galena showed an increase in BAF from lower pH (1.3 or

1.5) to higher pH (2.5 or 3.0). Native Pb was another sample to show the unexpected positive trend in BAF % change. All remaining 7 samples showed the expected decrease in BAF when comparing the lower pH to the higher pH. This supports the theory that a more acidic stomach will result in a higher bioavailability of a lead sample.

4.5. Fluid-to-Solid Ratio

In vivo studies show a correlation between sample amount ingested and subsequent lead bioavailability (Barltrop and Meek, 1975; Conrad and Barton, 1978; Aungst *et al.*, 1981; Dieter *et al.*, 1993; Cohen *et al.*, 1994; Schoof *et al.*, 1995; WHO, 1995). As the concentration of the dose increases, the body may exhibit a saturation response. Whether or not this saturation process is occurring with the uptake of Pb by the human system is very important in setting limits of acceptable environmental Pb exposure.

With the *in vitro* experiment, a response to changes in the fluid-to-solid ratio was tested for both Pb and As. Results showed an increase in bioavailability with an increase in fluid amount for the case of Pb (Figure 20). The same sample (NIST MS 2710) was run through the *in vitro* experiment at varying ratios of *sample size : solution amount*. As the ratio increased, the Pb concentration also increased. With an increase in Pb concentration, the bioavailability ultimately decreased about 11%. Arsenic however, exhibited the opposite response. As the concentration of arsenic in

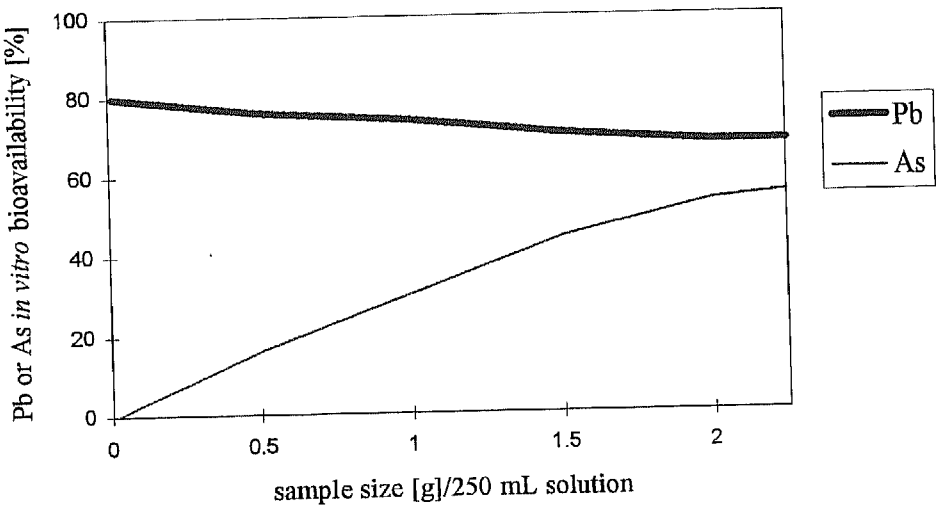


Figure 20. General trendline for changes in fluid:solid ratio.
Sample: NIST MS2710.

the same sample decreased, the bioavailability decreased a total of 71% (though results may overestimate As BAF due to As_{aq} values measured close to ICP-AES detection limit).

4.6. Particle Size Effects on Bioavailability

Dissolution of lead was examined as a function of sample particle size. When a Pb-solid is ingested by humans, it undergoes a reduction in particle size as atoms of Pb^{2+} are progressively released from the solid Pb-particle by ion exchange with H^+. Dissolution reactions in acid involve the binding of protons to the surface ions, weakening the critical bonds and detaching the metal into solution (Stumm and Morgan, 1981). As surface area becomes greater, there are more sites exposed to the surface; a greater number of sites translates to more rapid Pb release. According to the (modified) Noyes-Whitney dissolution rate law (Healy, 1984):

$$\frac{dC}{dt} = \frac{kDS}{Vh}(C_s - C)$$

where : C = concentration of dissociating metal in solution
k = proportionality constant
D = diffusion coefficient
S = surface area
V = solution volume
h = diffusion layer thickness around a particle
C_s = equilibrium solubility

an increase in surface area will lead to an increase in dissolution rate.

In vitro results (Figure 21) verified this relation between particle size and bioavailability/dissolution rates. The smaller particle size sample (larger surface area) generally corresponded to both an increase in absolute bioavailability of lead, and an increase in dissolution rates. Among the six lead phases studied, an average of a 5-fold increase in absolute bioavailability occurred when comparing the $< 38\ \mu m$ particle size to the $250\ \mu m$ - $150\ \mu m$ size range. The "less soluble" lead phases: anglesite, pyromorphite, and galena showed a stronger particle-size dependence than the more soluble phases. Anglesite exhibited a 13-fold increase in bioavailability with decreasing particle size; this was the largest increase of the 6 minerals tested. Pyromorphite showed a 6-fold increase; and galena showed a 4-fold increase.

Along with a stronger dependence on particle size, the "less soluble" lead phases (anglesite, pyromorphite, and galena) also exhibited a higher rate of dissolution that continued to rise even after the 1 hour stomach phase of the experiment, than did the more soluble lead phases (slag, cerussite, and lead oxide). The dissolution of the more soluble lead phases often reached an equilibrium within the first 20 minutes of the stomach phase of the experiment (Figure 21).

The more soluble minerals showed a weaker bioavailability dependence on particle size; cerussite (K_{sp} $PbCO_3 = 1.46 \times 10^{-14}$; Lide, 1994), and lead oxide showed a 3-fold and 2-fold increase in bioavailability with decreasing particle size, respectively. This weak dependence on particle size may indicate that a lead phase with an exceptionally high bioavailability in the stomach acid will retain a high bioavailability no matter what the particle size of the sample. For instance, lead oxide

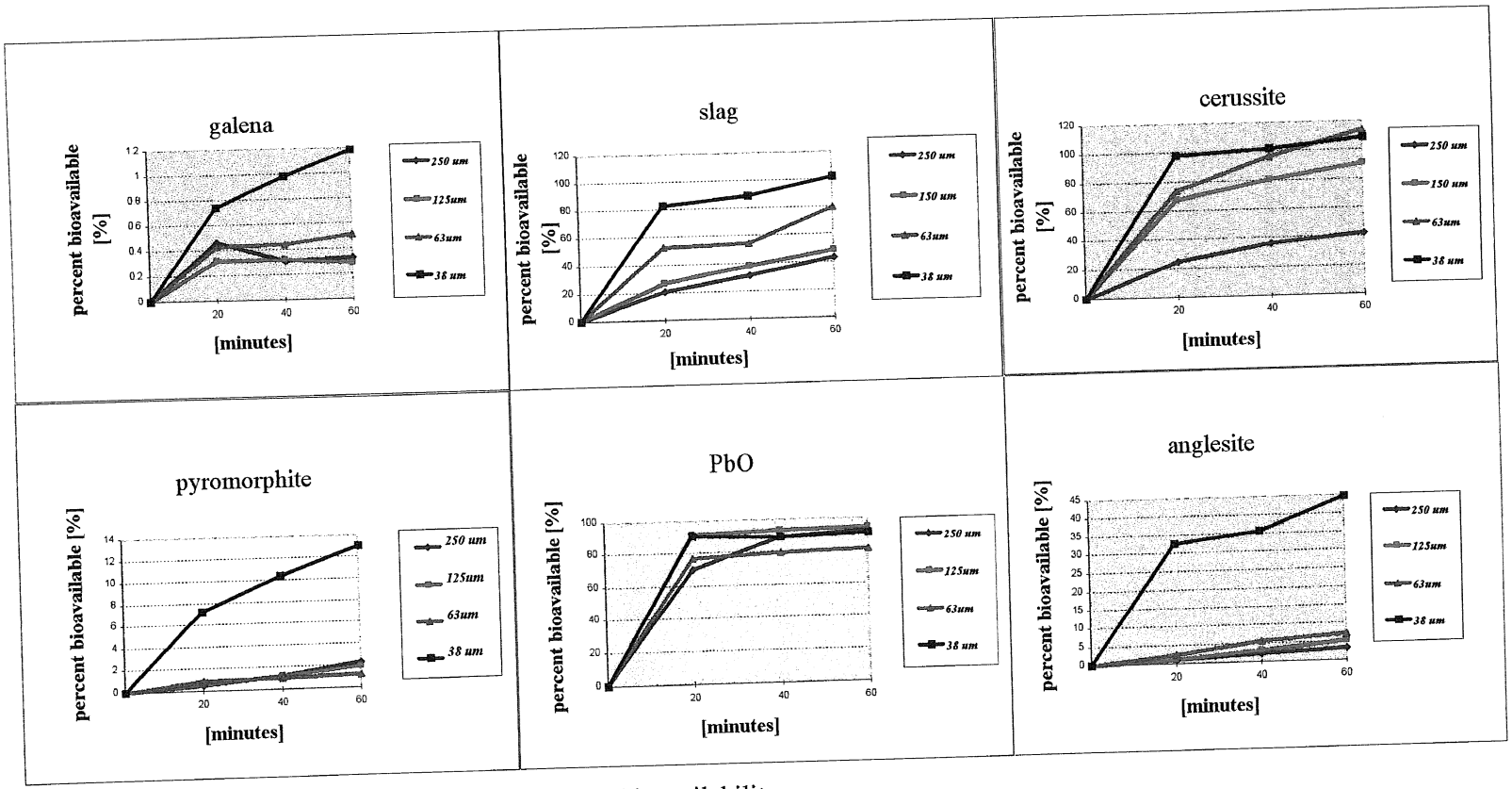


Figure 21. Effects of particle size on bioavailability.

will have a BAF of 90 - 100% if all sample characteristics are kept constant while the particle size is varied.

Small particle size can be a characteristic of either the original sample itself or the chemical reactions occurring in the low stomach pH. In both cases however, it is evident that ingestion of finer particles of Pb-bearing media is potentially more hazardous than ingestion of larger particles. Further *in vitro* research is recommended to be done on the particle size range between <63 μm and >38 μm, especially for pyromorphite, anglesite, and galena, which showed a gap in the results between that particle size range.

4.7. HCl vs. Stomach Solution

Lead and arsenic samples tested at a pH of 1.5, showed little difference between dissolution in stomach solution and dissolution in a HCl/deionized solution (Figures 22, 23 & 24). Anglesite showed a 13% decrease in Pb BAF for the HCl solution compared to the *in vitro* solution. Cerussite showed a 2% decrease in Pb BAF when comparing the stomach solution to the HCl solution. NIST MS 2710 also showed little difference between the two solutions for both Pb and As dissolution; the *in vitro* solution was 4% less bioavailable for Pb, and 6% less bioavailable than the HCl solution for As. When the higher stomach pH was tested however, the difference in Pb and As bioavailability was greater. For NIST MS 2710 in a solution of pH 2.8,

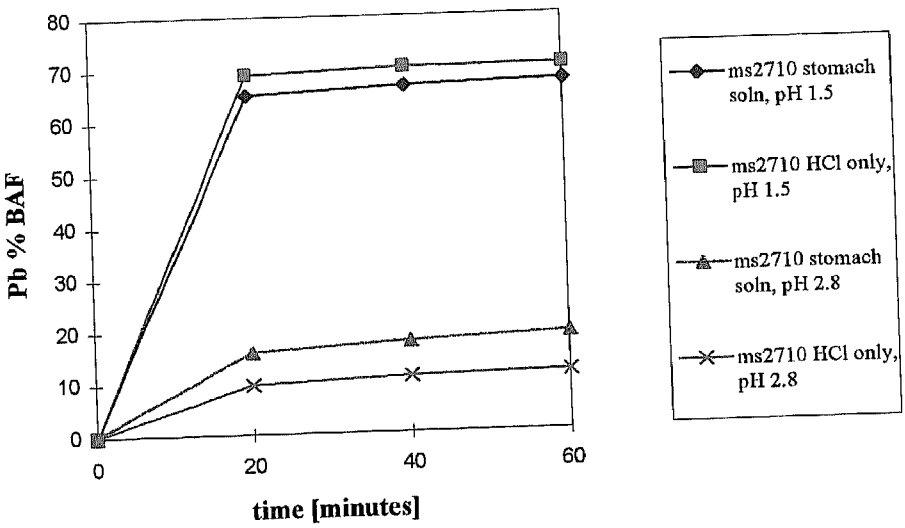


Figure 22. Comparison of stomach solution vs. HCl at varying pH's. Sample : MS 2710; results for Pb only.

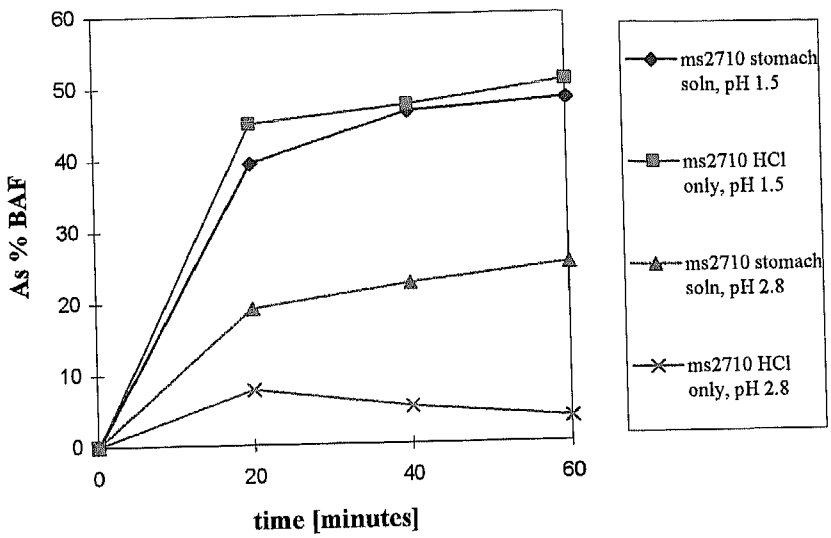


Figure 23. Comparison of stomach solution vs. HCl at varying pH's. Sample : MS 2710; results for As only.

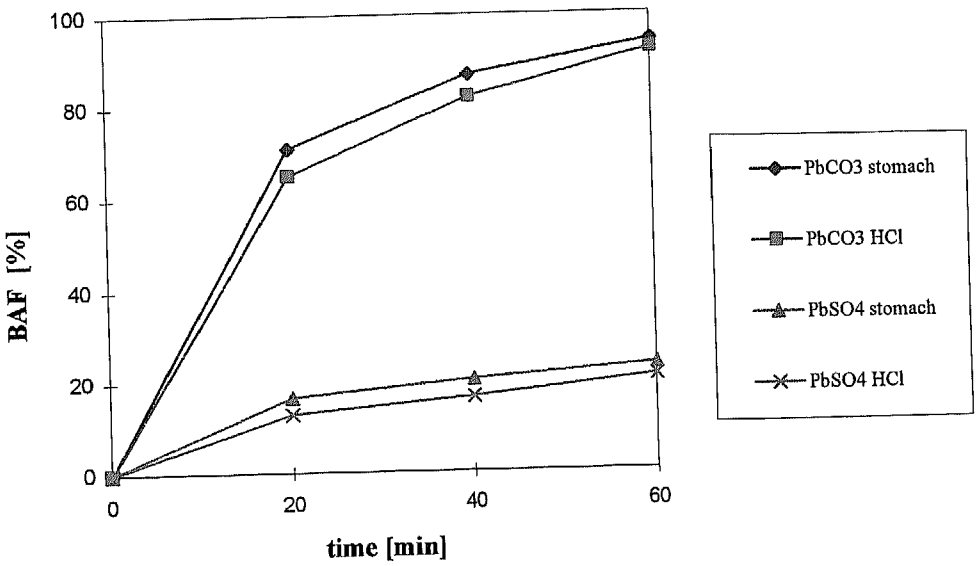


Figure 24. Stomach solution vs. HCl. Results for PbCO3 and PbSO4 only (pH 1.5).

the difference between the *in vitro* stomach solution and the HCl/deionized water solution was 42% less for Pb in HCl, and 84% less for As in HCl. Further research on samples at this higher pH is recommended to compare the potential differences between the stomach solution and the "HCl only" solution.

4.8. Intestinal Phase

Two Pb samples were run through the entire *in vitro* procedure: PbO, and MS 2710. The stomach phase was run at pH 1.5 for 1 h, and then the intestinal phase was continued for another 2.5 h at a pH of 7. Results showed the same general trend for both samples (Figure 25). For both PbO and MS 2710 the initial 20 minutes of the experiment resulted in the fastest overall rate of dissolution. After these 20 minutes, both samples' rates tended to plateau for the remainder of the 1 h stomach phase. The absolute rate increased again at the onset of the intestinal phase and continued at a moderate pace for the first 30 minutes of this phase. This 30 minute interval coincides with a gradual increase in gastrointestinal pH from the acidic conditions of the stomach (pH 1.5) to the neutral conditions of the intestine (pH 7). Once the neutral point was reached, the rates plateaued for the remainder of the intestinal phase. The lead oxide phase declined from 100% bioavailable in the stomach, to 67% bioavailable in the intestine; this corresponds to a 33% decrease in BAF. NIST MS 2710 declined from 74% bioavailable in the stomach, to 56% bioavailable in the intestine; corresponding to a 24% decrease in BAF for Pb only. Both samples

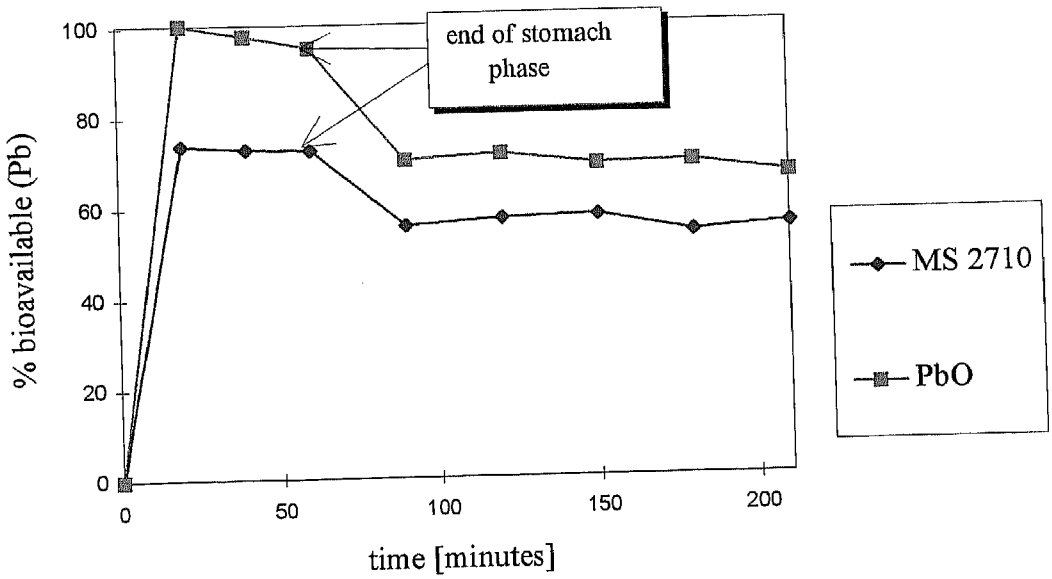


Figure 25. *In vitro* procedure: stomach and intestinal phase.

showed an average decrease of approximately 30% in Pb bioavailability from the initial acidic stomach phase through to the final (neutral) intestinal phase.

4.9. Effects of Food on Bioavailability

The addition of 10 mL of vitamin D-fortified milk to lead oxide did not significantly alter the expected bioavailability of PbO; it remained at the ~95% bioavailability mark (Figure 26). However, doubling this amount of milk (20 mL), decreased the BAF to 58% bioavailable which is much lower than the expected bioavailability of PbO. Doubling this amount once more (40 mL of milk) substantially decreased the bioavailability of PbO to 17%. The overall decrease from 10 mL addition to 40 mL addition resulted in an 82% reduction in the bioavailability of lead oxide. This reduction in bioavailability was not caused by dilution because the sample 250 mL volume was kept constant; however it may have been a result of an increase in solution pH associated with addition of milk since the solution pH was not kept constant (and did rise about 1.0 pH point at the maximum milk addition of 40 mL).

The results from food addition to the reaction vessel were inconclusive; no definite trends are seen in the data presented here (Figure 27). Further refinement of the *in vitro* method with solid foods involved is required to examine the effects on bioavailability from food presence in the stomach.

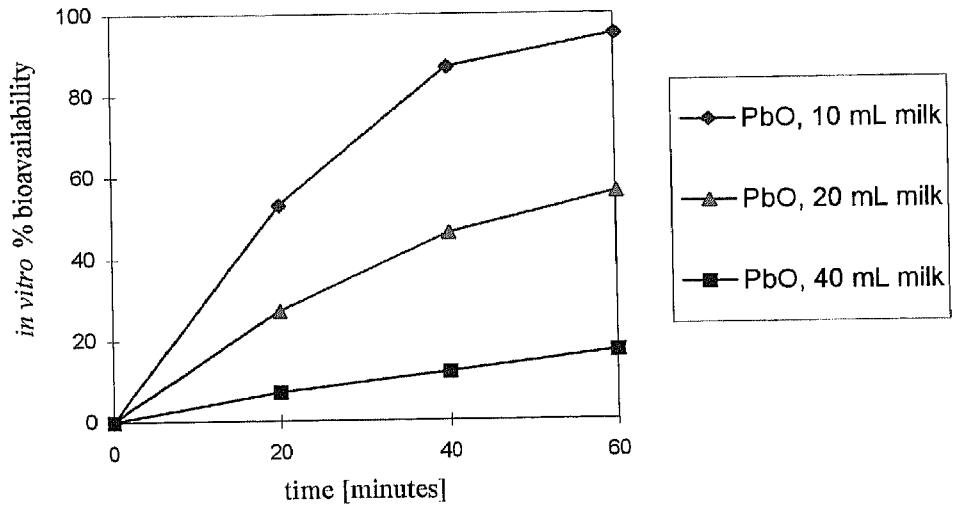


Figure 26. Effects of milk added to lead oxide (PbO) under the *in vitro* conditions described.

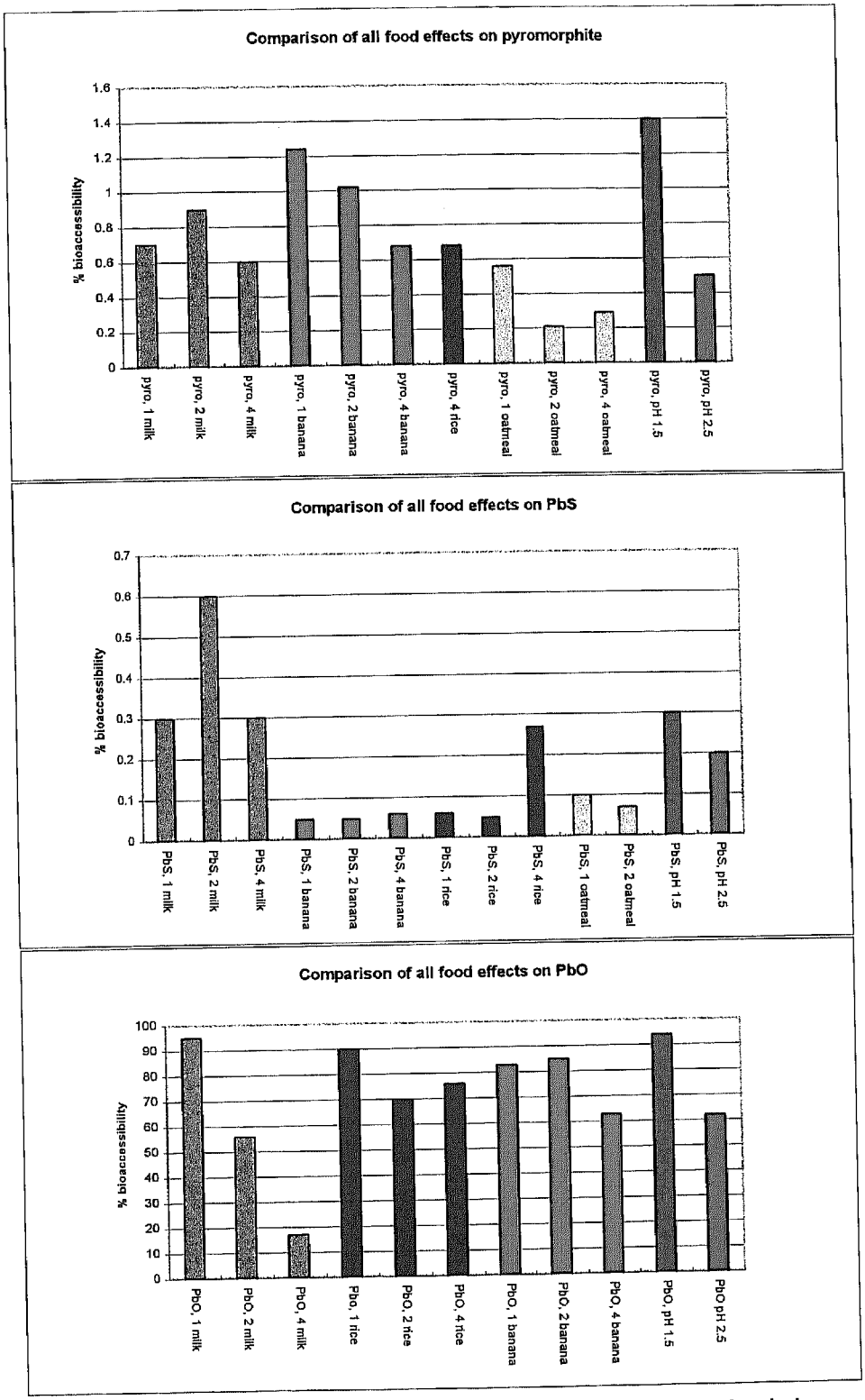


Figure 27. Effects of food (milk,rice,banana) added to lead samples (pyromorphite, galena, lead oxides). (Note: label e.g. "1 milk" refers to 10 mL of milk added to stomach solution)

V. DISCUSSION

5.1. Calibration

The relative percent difference of spiked samples and duplicates each demonstrate the reproducibility of the *in vitro* method for predicting bioavailability. A conservative default value for error bars on the percent bioavailability of any *in vitro* sample (using this proposed method) would be ± 10%, though many samples may fall closer to the ± 5% error range. The default uncertainty range must take into account various categories of potential error throughout the stages of the experiment. Examples of such potential error are: flaws in sample homogenization, dry sample weighing error, inaccurate total bulk metal value (XRF), stomach solution pH inconsistencies, pH probe inaccuracies, stomach solution preparation inconsistencies, fluctuations in rate of stirring, aqueous sampling contamination (removing or replacing volumes), reaction vessel or stirring rod surface contamination, final aqueous metal determination (ICP-AES), and individual user inconsistencies.

In addition to the good reproducibility of the *in vitro* method, the calibration of the method also proved to be successful, especially for the case of lead. With the use of draft EPA bioavailability values from an extensive swine-dosing study, the much less expensive and more controllable laboratory *in vitro* method was calibrated. A simple Cartesian plot of the EPA bioavailability value vs. the *in vitro* bioavailability value showed an almost one-to-one correlation between the two sets of data as shown by the r^2 values: 0.85 for Pb, and 0.86 for As. In the future, as more contaminated test samples are fed to the swine, more samples can be added to the regression line for the calibration of the *in vitro* method. Eventually this laboratory model may provide a more efficient and cost-effective means of estimating bioavailability than *in vivo* studies for predicting the bioavailability of soil lead in humans.

5.2. Sample Properties

The influence of mineral phase is one of the dominant sample characteristics that may affect subsequent bioavailability in humans. Bioavailability of a lead mineral in the gastrointestinal tract is controlled by several mineralogical conditions: solubility of the particular Pb-bearing phase in the stomach; encapsulation in an insoluble matrix; rinding of Pb grain by co-precipitation or alteration reactions during weathering; other surface effects such as adsorption and ion-exchange; interactions with organic phases; and dissolution kinetics of Pb bearing minerals (Bowie and

Thornton, 1985; Ruby *et al.*, 1996). Due to the possible variations in lead contaminated samples, one overall bioavailability value cannot be chosen to represent lead in all solid phases.

A compilation of bioavailability numbers from 13 mining communities showed galena to have a low bioavailability (Danse *et al.*, 1995). Similarly, numerous duplicate samples of galena run through this *in vitro* experiment confirm this finding (Medlin and Drexler, 1995). Healy *et al.*, (1982) suggest that galena is altered to $PbCl_2$ by reacting with the HCl in of the stomach. Lead chloride is much more soluble and thus a more readily available form of Pb, as can be noted by the solubility product constant for each Pb mineral (Mushak, 1991):

$$K_{sp}\,PbS = 3.4 \times 10^{-28}; \quad K_{sp}\,PbCl_2 \approx 10^{-4}$$

However, by microprobe examination of a post-*in vitro* galena (Drexler, personal comm., 1996), no alteration of galena particles was found in the sample. This hypothesis by Healy *et al.* (1982) therefore is not supported by *in vitro* (nor *in vivo*) findings.

Another lead mineral form, pyromorphite, found to contaminate the town of Derbyshire, England, did not show corresponding high PbB values in the people even though soil Pb levels were extremely elevated (Cotter-Howells and Thornton, 1991). Mineral bulk lead values may be very high among soils, and still the resulting PbB may be low. However, PbB cannot be used as a sole indicator of the bioavailability of lead; it may be the case that the exposure pathway between Pb and the people of the Derbyshire town has been blocked off, and the pyromorphite in the soil is of a

bioavailable form – it just does not have an open route to the people. On the other hand, if the soil was comprised of 10% galena instead of pyromorphite, it could still have a very low bioavailability even though the soil contained 100,000 mg kg^{-1} lead because galena is not a bioavailable lead mineral. If the soil was 10% lead oxide however, even though the bulk lead concentrations would be almost the same as in the soil with 10% galena, the lead oxide form of lead is extremely bioavailable and the PbB could reflect this.

In vitro studies of pure pyromorphite also showed a very low Pb bioavailability; but soils high in lead phosphates (e.g., Bingham soil, Table 6) were approximately 50% bioavailable by *in vitro* technique. The solubility product of pyromorphite ($K_{sp} = 10^{-84.4}$) indicates that the mineral is very insoluble. The difference between the *in vitro* bioavailability of these two forms of lead phosphate (crystalline and naturally occurring soil) is still an unanswered problem.

Besides galena and pyromorphite, other lead phases were tested *in vitro*. Cerussite and lead oxide both exhibited a relatively high bioavailability: 85 - 100% and 95 - 100% respectively. According to *in vivo* results, cerussite also produced a higher bioavailability than lead chromate when rats were dosed with lead paint (Clapp *et al.*, 1991). In another rat study, cerussite was absorbed 164%, and native lead was only 14% absorbed (Barltrop and Meek, 1979).

Anglesite, another mineral examined in this study, exhibited a relatively low *in vitro* BAF: 15 - 25%. Even less bioavailable than anglesite were galena (BAF: 0 - 1%) and pyromorphite (BAF: 1 - 5%). Lead-bearing iron and manganese oxides and

hydroxides (cave sediments) also resulted in a relatively low bioavailability: 0 - 1% and 16 - 18%, respectively.

In addition to studying the effects of mineral phase on bioavailability, the effect of sample particle size was also extensively studied by *in vitro*; results verified the theory that an increase in surface area will correspond to an increase in bioavailability. The question of whether or not a decrease in particle size will alter the bioavailability of a lead mineral is important because it is the small particles that tend to adhere to a child's hands (Duggan *et al.*, 1985). Common lead sources in the environment including paint, smelter emissions, wind-blown tailings, and mine wastes used as fill material, all have a common characteristic: their particle size is typically small.

The concentration of lead in dust is often greater than the concentration found in soils, partially due to the association of Pb with the clay-sized particles present in dust (Spittler and Feder, 1979; VanBorm *et al.*, 1988; Kendall, 1991). According to Kendall (1991) there is a very high correlation between the sample's highest Pb concentrations and smallest particle sizes (Figure 28). Overall, the small-sized particles (< 150 μm) from a smelter site in Butte, Montana, have 38% higher Pb concentrations than the total soil sample concentration. Similarly, VanBorm *et al.* (1988) studied samples from a smelter site in Belgium and concluded that by using the bulk concentration to represent the smallest size fraction (< 10 μm), the Pb concentration will be underestimated by at least a factor of 10.

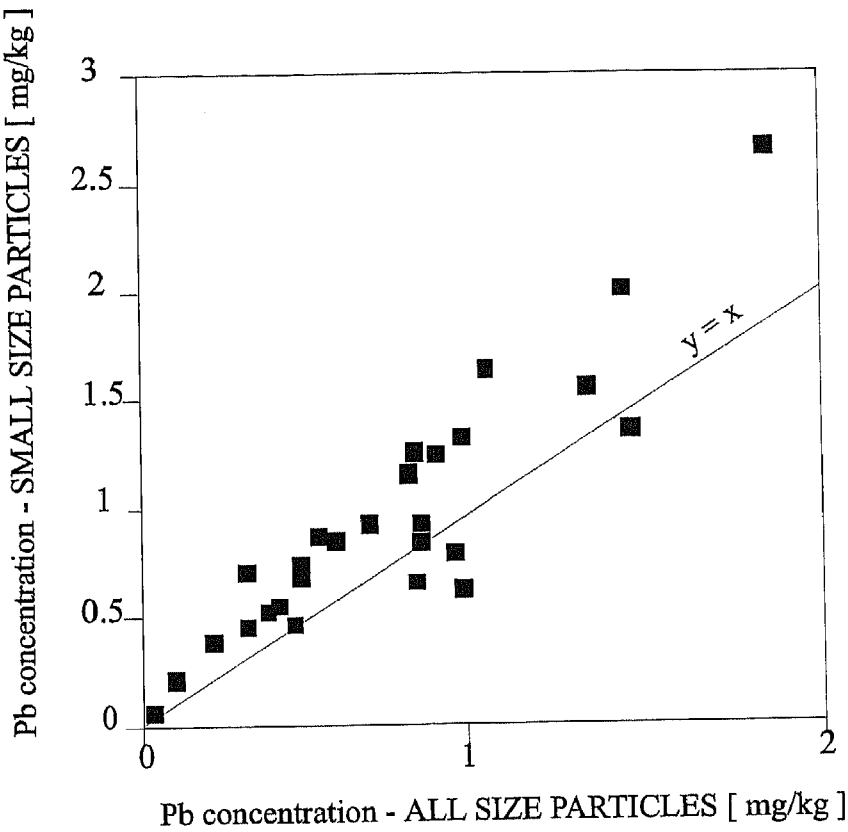


Figure 28. Comparison of bulk lead concentrations between the entire sample and only the small- sized particles (Kendall,1991).

In an *in vivo* study, lead dosed diets of 6 particle size ranges (from 6 to 197 μm) were fed to rats to examine the effects of particle size on Pb absorption (Barltrop and Meek, 1979). A five-fold enhancement of absorption was observed from the diet with lead particles of mean size 6 μm, compared with the 197 μm particle size diet (Figure 29). As the size of the metallic Pb particles decreases, the surface area of the sample increases, and so does the bioavailability. Similarly, by examining dissolutions of two different bulk particle size samples of galena in gastric fluid, Healy *et al.* (1982) found a strong correlation between small particle size and high Pb bioavailability. Although both samples reached a plateau after three hours in their experiment, it is possible that the larger particle size sample could outlast the residence time in the stomach and pass through the GI tract before becoming soluble (Healy *et al.*, 1982).

5.3. Physiological Properties

A number of changes in pH occur as material is moved through the human digestive tract. In addition to alterations in pH, there are important changes in motility, hormonal state (i.e., vitamin D), and enzyme secretion that may also affect bioavailability. Redox conditions also strongly influence metal speciation; metal absorption decreases with increasing pH due to complexation of the metal ion with the hydroxide ion (Cheng *et al.*, 1991). Leaching of heavy metal ions from soil and

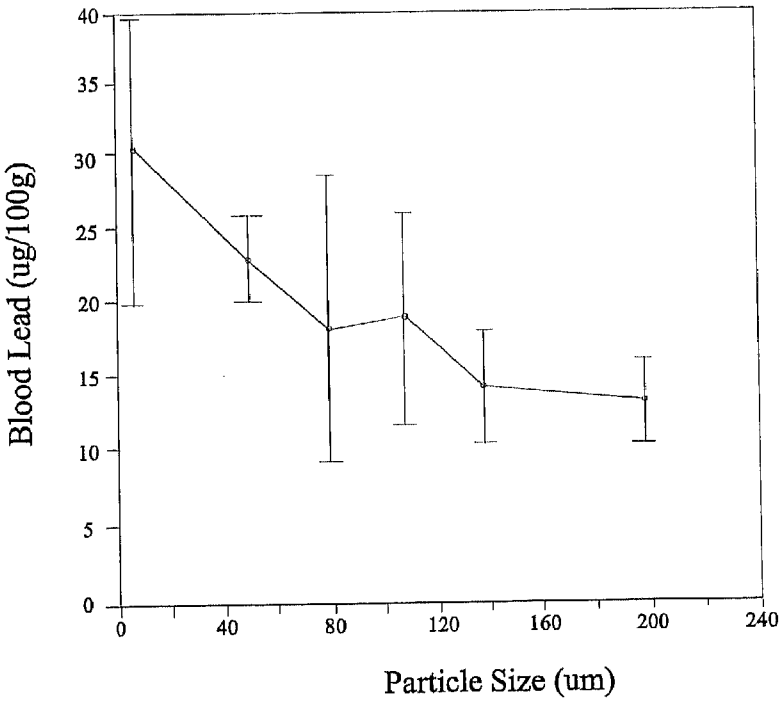


Figure 29. Effect of metallic lead particle size on blood Pb concentration (Barltrop and Meek, 1979).

other particulate matrices increases significantly when pH drops below 5 (Tessier *et al.*, 1979). Gastric acid is important in the mobilization of metals when contaminated soils are ingested because the gastric environment is often well below pH 5. In all cases, except for galena, *in vitro* results showed the expected increase in bioavailability with decreasing stomach pH. Galena showed a very slight decrease in bioavailability from higher pH to lower pH: 0.2% to 0.1% respectively, however this difference is not statistically significant.

The small intestine is the principal organ of absorption in the body, however *in vitro* results for the intestinal phase (Figure 25) show this value to underestimate the *in vivo* bioavailability value. Results from the stomach phase, on the other hand, consistently proved to more accurately reflect the *in vivo* results for Pb bioavailability. A possible explanation for this may be that all of the Pb soluble in the human stomach is completely absorbed in the intestine. Other researchers using a similar *in vitro* technique (Ruby *et al.*, 1996) have found the same results with regard to the intestinal phase underestimating *in vivo* results when calibrating with alternative *in vivo* experiments.

The presence of food in the digestive tract can theoretically decrease the bioavailability of a heavy metal (Rabinowitz *et al.*, 1980). *In vitro* results showed inconclusive evidence in support of this theory (Figure 25). Examples of parameters that may affect the absorption in the gastrointestinal tract are the presence of fat, milk, fiber, alcohol or other drugs (Cikrt *et al.*, 1994). The lack of food in the GI tract may also affect absorption by lowering the pH. Studies indicate that food may affect absorption in the GI tract from a time period of 2 hours before, until 3 hours after the

specified time of concern (James *et al.*, 1985). With a meal, the human stomach can reach pH 6 (Dressman *et al.*, 1993); while under fasting conditions the pH may be as low as 1 - 1.5. Gastrointestinal absorption can be as high as 70% in fasting humans or as low as 2 - 10% in feeding humans (Chaney *et al.*, 1989). However, an increase in dietary fat may also increase the amount of Pb absorbed (Barltrop and Meek, 1975).

In particular, milk may lower the bioavailability of lead by co-precipitation with Ca phosphates formed in the duodenum (Chaney *et al.*, 1989). Milk with a low lactose concentration and high level of calcium may provide protection against lead toxicity (Bushnell and DeLuca, 1983). Also, the vitamin D in milk may stimulate calcium and lead absorption (Ragan, 1983).

Another factor that can be considered when examining the bioavailability of a sample with the *in vitro* technique is the effect of changes in fluid-to-solid ratio. Many researchers have reported on a dose-response correlation for lead (Barltrop and Meek, 1975; Conrad and Barton, 1978; Aungst *et al.*, 1981; Dieter *et al.*, 1993; Cohen *et al.*, 1994; Freeman *et al.*, 1994; WHO, 1995). The *in vitro* results from this study show a "dose-like" response for lead, and an opposite response for arsenic.

In vivo studies with rats show a strong dose response for lead (Aungst *et al.*, 1981). This result may indicate that the toxic effects of lead may not be easily extrapolated from high levels of exposure to low, as they have been in Figure 19 for *NIST MS 2710*. Perhaps instead of extrapolating a linear curve to the lowest dose level (< 0.5g 250 mL^{-1}), it needs to be curved asymptotically at the y-axis. Bioavailability of low doses of exposure are particularly of interest for the subject who is chronically exposed to low levels of Pb.

VI. CONCLUSION

6.1. Summary

Lead comprises a major portion of the hazardous waste present in our environment. This naturally occurring heavy metal can be both an aid to industrial societies as well as a harmful contaminant. Levels have been set by the EPA to curtail the presence of this potentially hazardous metal, but these regulations may need to be adapted to fit site-specific risk assessments. Questions remain concerning the validity of modeling the complex relationships between blood-lead, environmental lead, and the bioavailability of lead. Many physical and chemical factors may influence the resulting bioavailability of a contaminated lead sample. Such influencing factors include: mineral speciation, encapsulation, co-precipitation, particle size, or alteration during weathering. Answers to these questions surrounding the bioavailability of lead may be gained by examination of specific contaminated samples by use of this *in vitro* experiment.

Studies using animals or humans to assess the extent of lead bioavailability are often costly and limited in their control. The proposed *in vitro* method presented

in this paper is a comparatively inexpensive, adaptable model for simulating the process of metal uptake and bioavailability in humans. This *in vitro* procedure is a calibrated, physiologically-based extraction technique used to study reactions of various lead-contaminated media in stomach solution. A human's stomach and digestive system are fashioned in a simple laboratory flask filled with synthetic stomach and intestinal solutions. As a necessary validation step, the *in vitro* test was calibrated to an appropriate *in vivo* model, swine. Results showed an almost one-to-one relation between animal and laboratory tests for lead. Arsenic results are somewhat less promising, and further testing is needed.

One of the main advantages this experiment offers is control of a desired variable such as: soil or waste type, particle size, mineral phase, food presence, and pH range of the stomach. All of these variables have been examined in this study. Results showed there is much variability between the bioavailability of lead from different contaminated media and lead sources. The range of estimated lead bioavailability was from non-bioavailable (0% BAF) to completely bioavailable (100% BAF). This phase-dependent character of lead bioavailability is important for establishing site-specific allowable levels for lead.

In some cases, particle size can be the limiting factor in lead bioavailability. The smaller particle size samples proved to have a greater initial bulk lead (e.g., smelter samples) as well as exhibit faster dissolution rates (due to increase in surface area). However, some samples were so bioavailable (e.g., lead oxide) that the sample particle size did not affect bioavailability. In general, mineral forms of galena,

pyromorphite, and anglesite had a relatively low bioavailability; while cerussite and lead oxide had a relatively high bioavailability.

The evaluation of food presence in the stomach did not appear to influence bioavailability estimates in our system, and further testing is needed in this area. The stomach pH value however, proved to be a highly sensitive parameter in our *in vitro* lead bioavailability system. Generally, the more acidic the stomach pH, the more likely lead will be solubilized and become available for uptake into the human system. While it is true that the intestine is the primary organ for lead absorption, the *in vitro* intestinal phase proved to underestimate the bioavailability of lead with respect to *in vivo* calibration studies.

6.2. Recommendations For Further Study

Now that the *in vitro* method has been calibrated by direct comparison with a scientifically valid *in vivo* system across a wide range of material types and solubilities, it can be used to qualify potentially hazardous lead-contaminated sites. Some high-lead concentrated sites may not need remediation for example, if the dominant lead phase is galena. Other sites may have relatively low concentrations of lead and may still need focused remediation efforts if contaminated by lead oxide or cerussite. Each site classified for clean-up efforts will have several factors influencing the bioavailability of lead. By use of the *in vitro* method to evaluate a particular site, clean-up levels can be better estimated.

The setup of this experiment focuses on designing a template for use in the field of bioavailability that could reliably predict levels of lead uptake if properly calibrated to a scientifically-valid *in vivo* model. If adequately calibrated, this *in vitro* model may plausibly be used to identify the bioavailability of other heavy metals such as: cadmium, arsenic, mercury, zinc, and selenium. When used in concert with other evaluation methods, the *in vitro* method could become a useful tool for setting remediation levels for contaminated sites (see Forbes *et al.*, 1989 for an excellent approach with Fe). Depending on the complex nature of a site, recommendations for study can be customized to fit the particular sites' needs. A complete and thorough evaluation of a contaminated site might include three complementary studies such as: an *in vitro* technique, an acceptable *in vivo* experiment, and a computer model for lead exposure. Initially, the *in vitro* technique could be used to screen the site quickly and inexpensively. Follow-up on the samples that do not pass acceptable limits of exposure can be supplemented by other testing methods. Computerized models for determining exposure to environmental lead, such as IEUBK (Integrated Exposure Uptake Biokinetic, USEPA, 1991; Chrostowski and Wheeler, 1992), and soil clean-up level models such as: truncated log normal (SEGH), Monte Carlo analysis (USEPA), or house-by-house approach (structural equation) (Bowers and Gauthier, 1994), may be used together with *in vitro* data and risk assessment models to estimate more precise clean-up levels for specific PbB levels from known exposure pathways. *In vivo* methods alone are often difficult to interpret, but if used in conjunction with *in vitro* data, a more accurate site evaluation may result.

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APPENDIX I.

CALIBRATION DATA.

COMPARISON OF EPA SWINE STUDY SAMPLES EXAMINED BY *IN VITRO* METHOD

Compilation of in vitro data vs. pig-fed samples from the EPA
All data are Relative Bioavailabilities (RBA's); all bulk Pb and As, and ICP values are ppm
EPA Pb - last updated: December 4,1996
EPA As - last updated: March 11, 1997
CU in vitro Pb and As - last updated: April 5, 1997

(1) Sample name:	Bingham Creek (Kennicott <3500,>3000), Salt Lake City, Utah		
sample type:	high lead soil		
PbB	30%		
total bulk Pb:	6330	***total bulk As :***	149
pig Pb bio% :	29%	***pig As bio % :***	31%

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	22.007	39%		x
60 min	22.448	39%		x

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	0.228	17%		x
60 min	0.234	17%		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	18.836	33		2.1
40 min	19.569	34		3.1
60 min	19.985	35		0.8

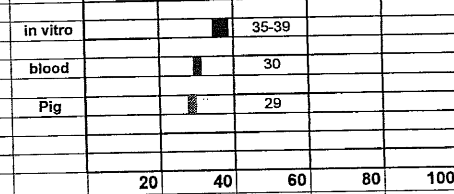
date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.188	14		x
40 min	0.221	17		x
60 min	0.195	15		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	18.006	32		x
40 min	19.684	35		x
60 min	19.734	35		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.161	12		x
40 min	0.233	17		x
60 min	0.26	19		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	19.2	34		x
40 min	20.351	36		x
60 min	20.827	37		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.173	13		x
40 min	0.214	16		x
60 min	0.203	15		x



(2) Sample name:	Bingham Creek (Kennicott <2500),Salt Lake City, Utah		
sample type:	low lead soil		
PbB	32%		
total bulk Pb:	1590	*total bulk As :*	51.2
pig Pb bio% :	31%	*pig As bio % :*	

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	6.807	48%		x
60 min	7.042	49%		x

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	0.17	37%		x
60 min	0.141	31%		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	5.817	41		2.1
40 min	6.295	44		28
60 min	6.497	45		0.7

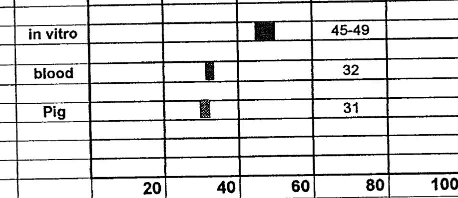
date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.195	43		x
40 min	0.191	42		x
60 min	0.191	42		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	5.894	41		x
40 min	6.501	45		x
60 min	6.567	46		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.176	38		x
40 min	0.158	34		x
60 min	0.18	39		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	6.165	43		x
40 min	6.498	45		x
60 min	6.713	47		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.176	38		x
40 min	0.199	43		x
60 min	0.76	38		x



(3) Sample name:	Jasper County HL Smelter (HL Smelter), Missouri			
sample type:	slag			
PbB	56%			
total bulk Pb:	10,800		***total bulk As :***	25
pig Pb bio% :	58%		***pig As bio % :***	

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	64.765	67%		x
60 min	67.903	70%		x

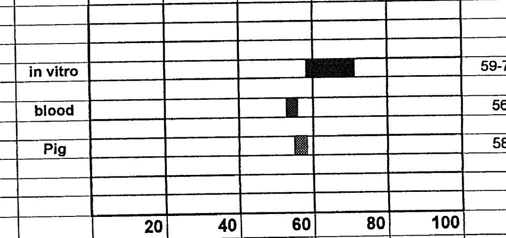
date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	0.129	57%		x
60 min	0.154	68%		x

date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	70.477	73%		x
60 min	74.34	76		x

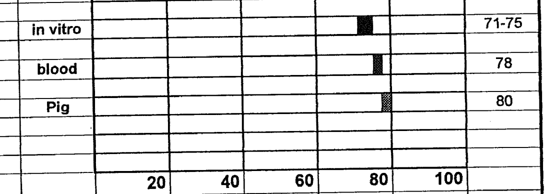
date:			pH:	
4/6/95			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	0.162	62%		x
60 min	0.154	64		x

date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	54.285	56		x
40 min	55.832	57		x
60 min	57.164	59		x

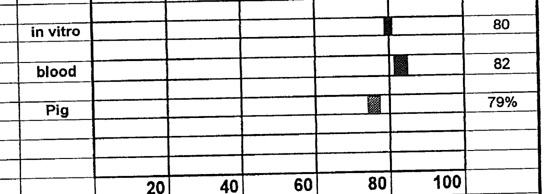
date:			pH:	
10/23/96			1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	0.24	107		x
40 min	0.15	67		x
60 min	0.098	45		x



(4) Sample name:			Jasper County LL Yard (LL Yard), Missouri							
sample type:			soil							
PbB			78%							
total bulk Pb:			4050			*total bulk As :*			11	
pig Pb bio% :			80%			*pig As bio % :*				
date:			pH:			date:			pH:	
1/9/96			1.2			2/7/96			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**			**sample**	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	25.806	71%		1.30%		**20 min**	0.093	94% (dl)		x
40 min	27.016	74%		2.70%		**40 min**	0.129	130% (dl)		x
60 min	27.372	75%		1.20%		**60 min**	0.105	106% (dl)		x
date:			pH:							
2/7/96			1.5							
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**							
20 min	21.045	58%		x						
40 min	24.686	68%		x						
60 min	25.829	71%		x						



(5) Sample name:			Jasper County HL Mill (HL Mill), Missouri	
sample type:			soil	
PbB			82%	
total bulk Pb:			6940	
pig Pb bio% :			79%	
date:			pH:	
1/9/96			1.2	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	45.757	73%		0.20%
40 min	49.709	80%		3.80%
60 min	50.23	80%		2.80%



(6) Sample name:	Murray Smelter (MHA-A36), Salt Lake City, Utah		
sample type:	slag		
PbB	55%		
total bulk Pb:	11,700	***total bulk As :***	710
pig Pb bio% :	53%	***pig As bio % :***	53%

Pb

date:		pH:	
3/22/95		1.3	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	x	x	
40 min	56.132	54%	1.5
60 min	68.158	66%	0.5

date:		pH:	
3/23/95		1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	x	x	x
40 min	69.824	67%	x
60 min	74.354	72%	x

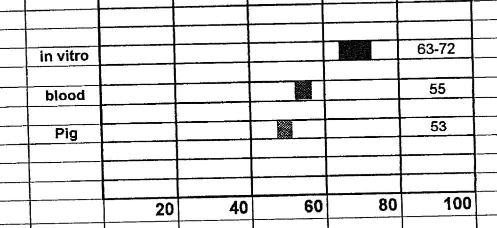
date:		pH:	
1/10/96		1.2	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	53.121	50%	3%
40 min	60.906	58%	2.50%
60 min	67.221	64%	5.30%

date:		pH:	
1/11/96		1.1	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	48.289	46%	x
40 min	61.244	58%	x
60 min	66.387	63%	x

As

date:		pH:	
3/22/95		1.3	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	x	x	x
40 min	2.62	42%	x
60 min	3.64	58%	x

date:		pH:	
3/23/95		1.5	
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	x	x	x
40 min	3.626	58%	x
60 min	3.826	61%	x



(7) Sample name:	Midvale slag (Midvale 1), Salt Lake City, Utah
sample type:	slag
PbB	20%
total bulk Pb:	7895
pig Pb bio% :	17%
total bulk As :	710
pig As bio % :	14%

date:			pH:	
1/11/96			1.2	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	9.103	13%		x
40 min	10.1	14%		x
60 min	11.986	17%		x

date:			pH:	
2/7/96			1.3	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.877	14%		x
40 min	1.077	17%		x
60 min	1.176	18%		x

date:			pH:	
2/7/96			1.3	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	1.197	2%		x
40 min	6.024	8%		x
60 min	7.872	11%		x

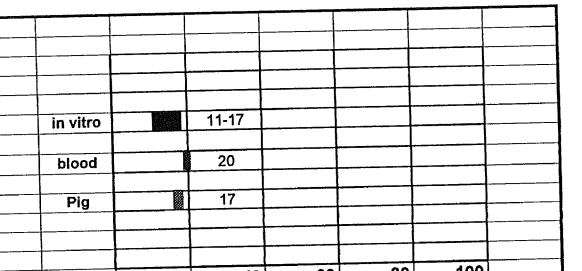
(8) Sample name:	Leadville Residential Composite (8-35690/-91/-92), Colorado
sample type:	lead soil
PbB	71%
total bulk Pb:	7510
pig Pb bio% :	74%
total bulk As :	11
pig As bio % :	bad

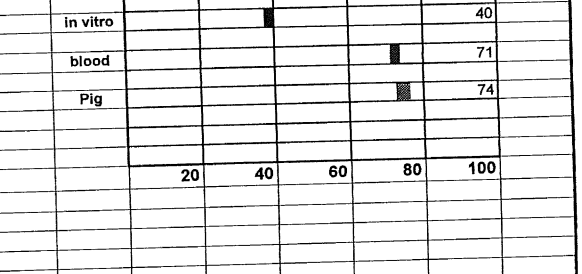
date:			pH:	
12/29/94			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	x	x		x
40 min	24.3	36%		x
60 min	26.901	40%		x

date:			pH:	
12/29/94			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	x	x		x
40 min	0.031	31% (dl)		x
60 min	0.022	22% (dl)		x

date:			pH:	
12/29/94			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	x	x		x
40 min	26.5	39%		x
60 min	26.899	40%		x

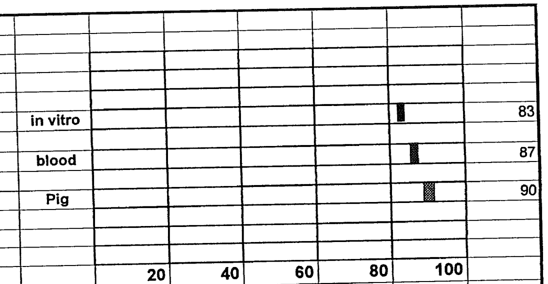
date:			pH:	
12/29/94			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	x	x		x
40 min	0.228	12%		x
60 min	0.243	13%		x





(9) Sample name:	Leadville Fe/Mn PbO (Lead Mn),California Gulch, Colorado			
sample type:	mining soil			
PbB	87%			
total bulk Pb:	4320		***total bulk As :***	110
pig Pb bio% :	90%		***pig As bio % :***	42%

date:			pH:	
1/11/96			1.1	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	28.007	72%		6%
40 min	31.287	81%		3.50%
60 min	32.247	83%		3%



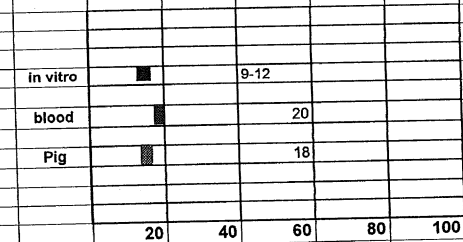
(10) Sample name:	Leadville AV Smelter Slag (Arkansas Valley; WQ <250), Colorado			
sample type:	slag			
PbB	20%			
total bulk Pb:	10,600		***total bulk As :***	1050
pig Pb bio% :	18%		***pig As bio % :***	16%

date:			pH:		date:			pH:	
1/4/95	throw out		2.5		1/4/95			2.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**		**sample**	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x	**20 min**	x	x		x
40 min	1.243	1.30%		x	**40 min**	1.653	17%		x
60 min	1.699	1.70%		x	**60 min**	1.887	20%		x

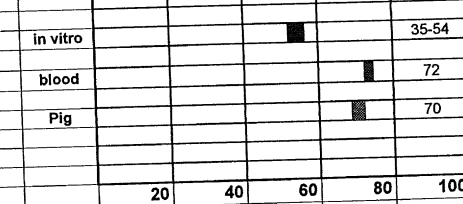
date:			pH:		date:			pH:	
3/22/95			1.3		3/22/95			1.3	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**		**sample**	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x	**20 min**	x	x		x
40 min	7.478	8%		x	**40 min**	1.826	19%		x
60 min	11.468	12%		x	**60 min**	1.866	20%		x

date:			pH:	
3/22/95			1.3	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**	
20 min	x	x		x
40 min	6.746	7%		x
60 min	8.746	9%		x

date:			pH:		date:			pH:	
3/25/95			1.5		3/25/95			1.5	
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**		**sample**	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**	
20 min	x	x		x	**20 min**	x	x		x
40 min	5.922	6%		x	**40 min**	1.678	18%		x
60 min	10.634	11%		x	**60 min**	2.112	20%		x



(11) Sample name:		Palmerton Location 2 (Palmerton 2, Pom 2), New Jersey			
sample type:		soil			
PbB		72%			
total bulk Pb:		3230		*total bulk As :*	110
pig Pb bio% :		70%		*pig As bio % :*	52%



date:			pH:	
1/9/96			1.2	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	11.462	39%		0.65%
40 min	13.842	48%		1.40%
60 min	15.135	52%		3.30%

date:			pH:	
2/7/96	throw out		1.4	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	7.913	27%		x
40 min	9.313	32%		x
60 min	10.252	35%		x

date:			pH:	
2/7/96			1.4	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.118	12%		x
40 min	0.12	12%		x
60 min	0.162	16%		x

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	12.32	43		1.7
40 min	14.357	49		3.1
60 min	15.68	54		1.8

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.113	11		x
40 min	0.158	16		x
60 min	0.139	14		x

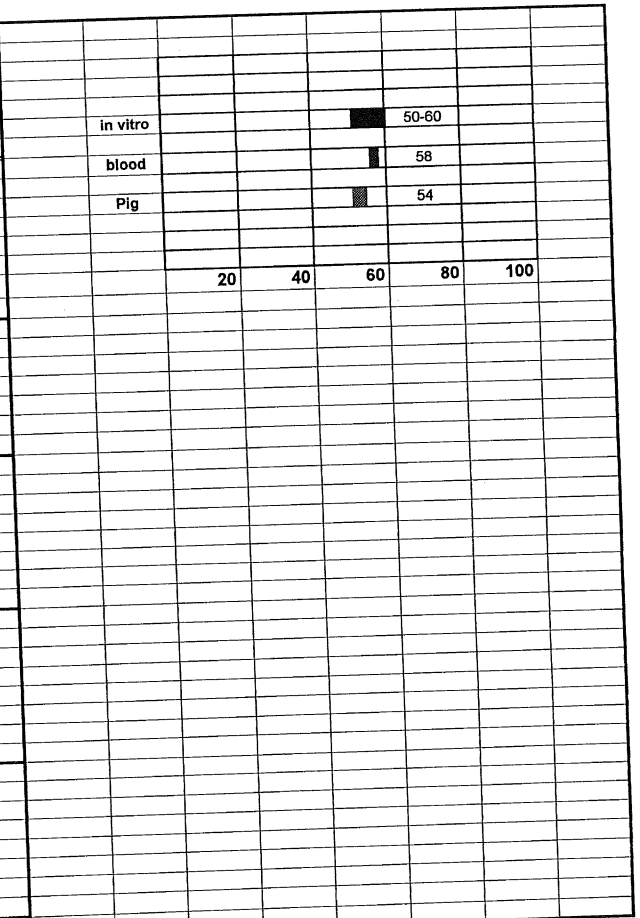
date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	12.054	42		x
40 min	14.568	50		x
60 min	15.742	54		x

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.079	8		x
40 min	0.135	14		x
60 min	0.169	17		x

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	12.131	42		x
40 min	14.211	49		x
60 min	15.723	54		x

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.101	10		x
40 min	0.15	15		x
60 min	0.161	16		x

(12) Sample name:			Palmerton Location 4 (Palmerton 4, Pom 4), New Jersey										
sample type:			soil										
PbB			58%										
total bulk Pb:			2150				*total bulk As :*			134			
pig Pb bio% :			54%				*pig As bio % :*			70%			
date:			pH:				date:			pH:			
1/10/96			1.2				2/7/96			1.4			
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)				sample	ICP	% bio (As)	rpd (spike 20 ppm)			
20 min	8.153	42%			15%		20 min	0.19	16%		x		
40 min	10.319	53%			20%		40 min	0.312	26%		x		
60 min	11.545	60%			24%		60 min	0.327	27%		x		
date:			pH:				date:			pH:			
2/7/96	throw out		1.4				10/23/96			1.5			
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)				sample	ICP	% bio (As)	rpd (spike 20 ppm)			
20 min	2.925	15%		x			20 min	0.263	22		x		
40 min	3.972	20%		x			40 min	0.378	31		x		
60 min	4.71	24%		x			60 min	0.449	37		x		
date:			pH:				date:			pH:			
10/23/96			1.5				10/23/96			1.5			
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)				sample	ICP	% bio (As)	rpd (spike 20 ppm)			
20 min	7.684	40			10		20 min	0.281	23		x		
40 min	9.63	50			9		40 min	0.387	32		x		
60 min	11.211	50			7		60 min	0.379	31		x		
date:			pH:				date:			pH:			
10/23/96			1.5				10/23/96			1.5			
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)				sample	ICP	% bio (As)	rpd (spike 20 ppm)			
20 min	7.622	39		x			20 min	0.269	22		x		
40 min	9.579	50		x			40 min	0.34	28		x		
60 min	11.265	58		x			60 min	0.375	31		x		
date:			pH:										
10/23/96			1.5										
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)										
20 min	7.677	40		x									
40 min	9.875	51		x									
60 min	11.063	57		x									



(13) Sample name:	Columbia 33 & 18 - human, Bunker Hill, Idaho		
sample type:	soil, Pb barite and sulfate		
total bulk Pb:	2924		
human	23-36	ABA	

date:			pH:	
1/8/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	15.987	61%		0.10%
40 min	17.219	65%		1.80%
60 min	17.849	68%		0.40%

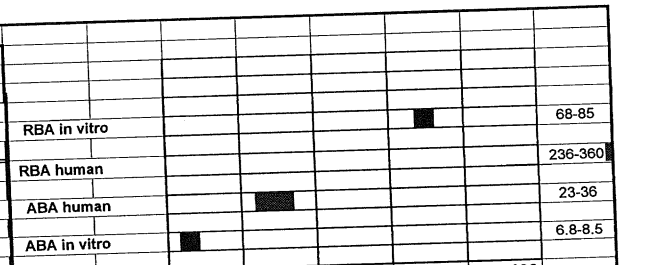
date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	15.941	61		x
40 min	17.845	68		x
60 min	19.124	72		x

date:			pH:	
1/8/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	17.126	65%		0.70%
40 min	18.578	71%		3.60%
60 min	19.162	73%		4.80%

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	16.681	63		x
40 min	18.75	71		x
60 min	19.026	72		x

date:			pH:	
10/24/96			1.3	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	19.852	75		x
40 min	21.217	81%		x
60 min	22.278	85%		x

date:			pH:	
10/24/96			1.3	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	18.141	69		x
40 min	19.52	74		x
60 min	20.218	77		x



(14) Sample name:		Leadville + paint					
sample type:		soil + NIST paint					
PbB		82%		*total bulk As :*		71	
total bulk Pb:		8350		*pig As bio % :*		not done	
pig Pb bio% :		80%					

date:			pH:	
2/8/96			1.2	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	45.974	61%		0.80%
40 min	48.216	64%		2.70%
60 min	48.688	65%		2.00%

date:			pH:	
2/8/96			1.2	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.027	4.20%		x
40 min	0.054	8.50%		x
60 min	0.059	9.20%		x

date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	50.339	67		x
40 min	51.915	69		x
60 min	52.618	70		x

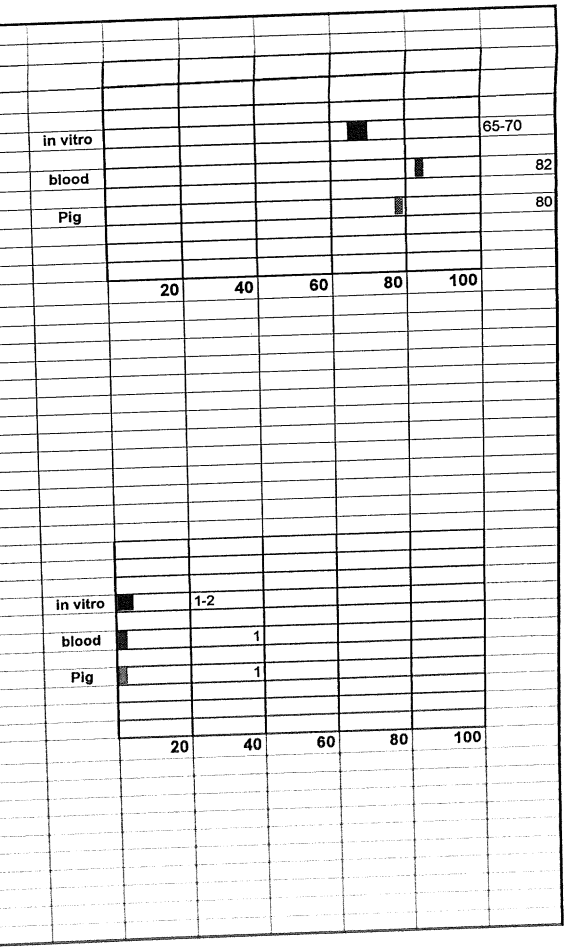
date:			pH:	
10/23/96			1.5	
sample	ICP	% bio (As)	rpd (spike 20 ppm)	
20 min	0.06	9.4		x
40 min	0.057	8.1		x
60 min	0.056	8.8		x

(15) Sample name:		Leadville + PbS					
sample type:		soil					
PbB		1%					
total bulk Pb:		11200					
pig Pb bio% :		1%					

date:			pH:	
2/8/96			1.2	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	0.591	0.60		7%
40 min	0.754	0.70		5.90%
60 min	0.884	0.90		6.80%

date:			pH:	
10/23/96			1.3	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	1.127	1.1		4.6
40 min	1.547	1.5		4.1
60 min	1.944	1.9		5.7

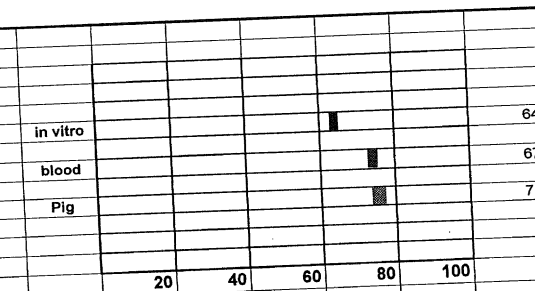
date:			pH:	
10/23/96			1.3	
sample	ICP	% bio (Pb)	rpd (spike 20 ppm)	
20 min	0.985	1		x
40 min	1.543	1.5		x
60 min	2.095	2.1		x



(16) Sample name:	New Murray, Salt Lake City, Utah		
sample type:	soil		
PbB	67%	***total bulk As :***	365
total bulk Pb:	3200	***pig As bio % :***	38%
pig Pb bio% :	71%		

date:			pH:
2/9/96			1.2
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	15.76	55%	3.90%
40 min	17.903	62%	4.20%
60 min	18.364	64%	5.20%

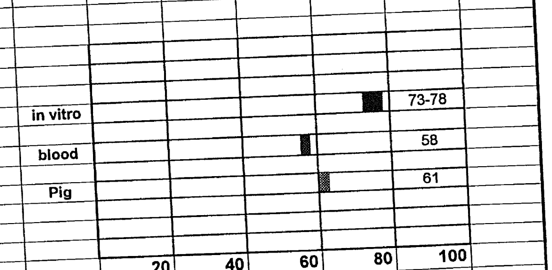
date:			pH:
2/9/96			1.2
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.318	39%	x
40 min	0.366	45%	x
60 min	0.386	47%	x



(17) Sample name:	Smuggler, Aspen Residential, Colorado(Aspen 3870, Aspen RW008)		
sample type:			
PbB	58	***total bulk As :***	17
total bulk Pb:	3870	***pig As bio %***	57%
pig Pb bio% :	61%		

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	24.556	71.00%	x
40 min	26.586	76.00%	x
60 min	27.362	78%	x

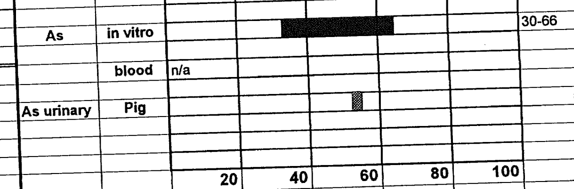
date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.105	69	x
40 min	0.214	140	x
60 min	0.143	94	x



date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	22.173	64	x
40 min	24.391	70	x
60 min	25.245	73	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.135	88	x
40 min	0.109	71	x
60 min	0.105	69	x

(18) Sample name:	Grant's Farm, Silver Bow Creek, Montana (SB-03, SB-05)		
sample type:	As soil		
total bulk Pb:	130	***total bulk As :***	181
pig Pb bio% :		***pig As bio%***	54%



date:			pH:
8/12/96			1.3
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	1.117	16.00%	x
40 min	0.798	12.00%	x
60 min	0.82	10%	x

date:			pH:
8/12/96			1.3
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.188	68	x
40 min	0.144	49	x
60 min	0.118	50	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.231	20	x
40 min	0.197	17	x
60 min	0.177	15	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.327	20	x
40 min	0.467	29	x
60 min	0.514	32	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.122	10	x
40 min	0.13	11	x
60 min	0.139	12	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.366	23	x
40 min	0.427	26	x
60 min	0.489	30	x

date:			pH:
8/12/96			1.3
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.152	13.00%	x
40 min	0.146	13.00%	x
60 min	0.182	16%	x

date:			pH:
8/12/96			1.3
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.936	58	x
40 min	1.005	62	x
60 min	1.082	66	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.186	16	x
40 min	0.243	21	x
60 min	0.277	24	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.546	34	x
40 min	0.979	44	x
60 min	0.716	49	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.183	16	x
40 min	0.192	16	x
60 min	0.2	17	x

date:			pH:
10/24/96			1.4
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.529	33	x
40 min	0.635	39	x
60 min	0.758	47	x

date:			pH:
2/1/97			1.5
sample	**ICP**	**% bio (Pb)**	**rpd (spike 20 ppm)**
20 min	0.054	4.6	x
40 min	0.1	8.5	x
60 min	0.076	6.5	x

date:			pH:
2/1/97			1.5
sample	**ICP**	**% bio (As)**	**rpd (spike 20 ppm)**
20 min	0.395	24	x
40 min	0.508	31	x
60 min	0.649	40	x